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DERMAL EYE AND ORAL TOXICOLOGICAL EVALUATIONS(U)

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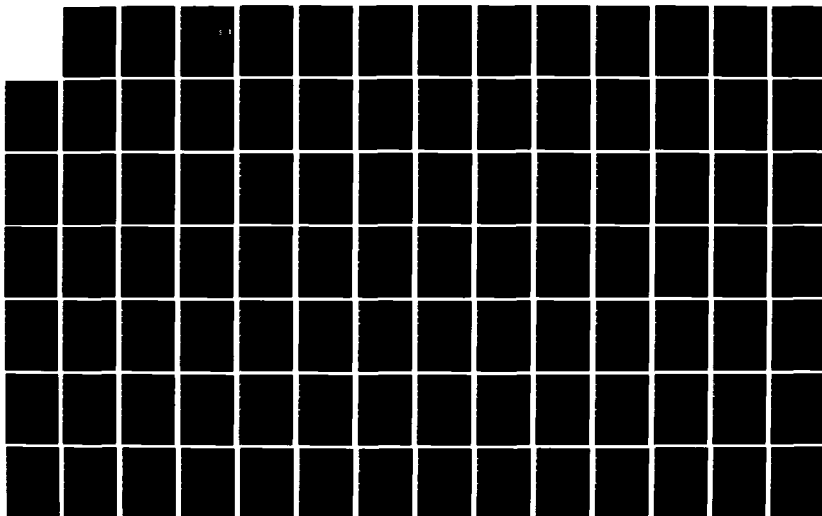
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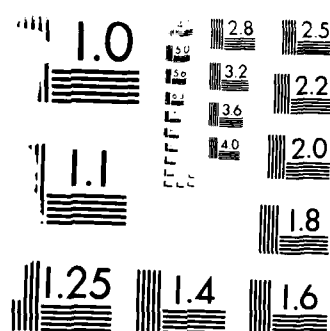
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BSC Project Number 11357

DERMAL, EYE, AND ORAL
TOXICOLOGICAL EVALUATIONS

PHASE II REPORT

PREPARED BY:

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February 1986

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) All five of the powdered test samples (11357-8,9,12,13,14)* were evaluated for dermal and eye irritation and oral toxicity. Dermal Irritation scores ranged from 0 to 0.08 with sample 11357-12 causing no irritation. Eye irritation ranged from non-irritating (11357-8) to moderately irritating (11357-13). When screened for oral toxicity, samples 11357-8,9, and 12 had oral LD ₅₀ values of greater than 5000 mg/kg, and samples 11357-13 and 14 had combined (male and female) LD ₅₀ values of greater than 3300 mg/kg. (continued next page)		

20. Abstract (continued)

The three materials tested for dermal toxicity (11357-8,9,12) had LD₅₀ values of greater than 2000 mg/kg body weight. Further testing of sample 11357-9 in the Two Week Multiple Dose Dermal Toxicity Study using dose levels of 1000, 200 and 50 mg/kg revealed no definitive increase in severity of skin lesions with increasing dose levels. It was also determined that sample 11357-9 was not a dermal sensitizer.

*Chemical Code Names

<u>BSC Code Number</u>	<u>Chemical Name</u>
11357-8	CI Solvent Green 3/CI Solvent Yellow 33 (70.9/24.1)
11357-9	CI Solvent Yellow 33
11357-12	1-acetyl-3,5,7-trinitro-1,3,5,7-octahydrotetrazocine (SEX)
11357-13	Copper-Zinc Coated Powder
11357-14	Copper-Zinc Powder

19. Key Words (continued)

1-acetyl-3,5,7-trinitro-1,3,5,7-octahydrotetrazocine (SEX)

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FOREWORD

This project was sponsored by the U.S. Army Medical Research and Development Command and the Army Materiel Command. Citation of commercial organizations and trade names in this report does not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

Animal Care Program Certification

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Uses of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

For I. A. Muni
Elliot B. Gordon, Ph.D.
Director of Animal Toxicology

Good Laboratory Practice Certification

All tests were conducted according to the protocol. All changes in the protocol have been documented. All reported results were inspected and found to accurately reflect the original data. These studies were performed according to the Good Laboratory Practice Regulations of the FDA (21 CFR 58.1-58.219, 1979).


Indu A. Muni, Ph.D.
Principal Investigator

Quality Assurance Inspection Statement

The study report was reviewed by ABC Quality Assurance Unit. Q.A. Unit also performed audits at different phases of these studies as shown in Appendix F and findings were reported to the Study Director and management.

F. U. [Signature]
Quality Assurance Specialist Date

TABLE OF CONTENTS

	<u>Page No.</u>
REPORT DOCUMENTATION PAGE	
FOREWORD	
EXECUTIVE SUMMARY	1
INTRODUCTION	5
MATERIALS AND METHODS	6
1. Test Materials	6
2. Animals	8
3. Experimental Design	8
4. Study Elements	10
Dermal Irritation	10
Eye Irritation	10
Oral Toxicity	10
Dermal Toxicity (Single Dose and Two-Week Multiple Dose)	11
Delayed-Type Contact Sensitization	11
5. Histopathology	12
RESULTS	13
Primary Dermal Irritation Test	13
Primary Eye Irritation Studies	21
Acute Dermal Toxicity Studies	28
Single Dose Oral Toxicity Studies	34
Acute Oral LD50 Determination	41
Copper-Zinc coated powder	41
Copper-Zinc powder	45
Two Week Multiple Dose Dermal Toxicity	50
CI Solvent Yellow 33	50
Delayed-Type Contact Sensitization Study	63
CI Solvent Yellow 33	63

TABLE OF CONTENTS (Continued)

Page No.

TABLES

Experimental Design Table	9
Legend for Tables 1-5	15
1 - Primary Dermal Irritation Study of CI Solvent Green 3/CI Solvent Yellow 33	16
2 - Primary Dermal Irritation Study of CI Solvent Yellow 33	17
3 - Primary Dermal Irritation Study of SEX	18
4 - Primary Dermal Irritation Study of Copper-Zinc coated powder	19
5 - Primary Dermal Irritation Study of Copper-Zinc powder	20
6 - Primary Eye Irritation Study of CI Solvent Green 3/CI Solvent Yellow 33	23
7 - Primary Eye Irritation Study of CI Solvent Yellow 33	24
8 - Primary Eye Irritation Study of SEX	25
9 - Primary Eye Irritation Study of Copper-Zinc coated powder	26
10 - Primary Eye Irritation Study of Copper-Zinc powder	27
11 - Acute Dermal Toxicity Study of CI Solvent Green 3/CI Solvent Yellow 33	31
12 - Acute Dermal Toxicity Study of CI Solvent Yellow 33	32
13 - Acute Dermal Toxicity Study of SEX	33
14 - Single Dose Oral Toxicity Study of CI Solvent Green 3/CI Solvent Yellow 33	36
15 - Single Dose Oral Toxicity Study of CI Solvent Yellow 33	37
16 - Single Dose Oral Toxicity Study of SEX	38
17 - Single Dose Oral Toxicity Study of Copper-Zinc coated powder	39
18 - Single Dose Oral Toxicity Study of Copper-Zinc powder	40
19 - Acute Oral LD50 Determination in F344 Rats (Copper-Zinc coated powder)	43
20 - Acute Oral LD50 Determination in F344 Rats (Copper-Zinc powder)	47
21-27 - Two Week Multiple Dose Dermal Toxicity Study in Rabbits of CI Solvent Yellow 33	
21 - Dose Level: 1000 mg/kg	56
22 - Dose Level: 200 mg/kg	57
23 - Dose Level: 50 mg/kg	58
24 - Clinical Signs	59
25 - Necropsy Findings	60
26 - Necropsy Findings	61
27 - Necropsy Findings	62

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TABLE OF CONTENTS (Continued)

Page No.

28-29 - Type Contact Sensitization Study of CI Solvent Yellow 33

28 - Body Weight (grams)	64
29 - Observations: Erythema/Edema	65

FIGURES

1 - Dose Response Curve for Copper-Zinc powder for Female F344 Albino Rats-Acute Oral LD ₅₀ Determination	49
--	----

APPENDICES

APPENDIX A. Test Methods for Dermal and Eye Irritation Studies	66
APPENDIX B. Test Method for Oral Toxicity Studies	72
APPENDIX C. Test Method for Acute Dermal Toxicity Studies	73
APPENDIX D. Test Method for Repeated Dose Dermal Toxicity Study	74
APPENDIX E. Test Method for Delayed-Type Contact Sensitization Study	75
APPENDIX F. Protocol Amendments and Deviations ^(a)	76
APPENDIX G. Quality Assurance Inspections	85
DISTRIBUTION LIST	89

EXECUTIVE SUMMARY

This program was designed to study the acute toxicity of five chemical substances. The objective was to compare the toxicity of these test materials. These safety data would then be reviewed to permit a reasoned selection of the most appropriate materials for use by the Army. The test materials were coded as BSC # 11357-X, where X equals 8, 9, 12, 13 and 14. The following is a list of the test materials with the corresponding BSC Code #:

<u>BSC Code #</u>	<u>Chemical Name</u>
11357-8	CI Solvent Green 3/CI Solvent Yellow 33 (70.9/24.1)
11357-9	CI Solvent Yellow 33
11357-12	1-acetyl-3,5,7-trinitro-1,3,5,7- octahydrotriazocine (SEX)
11357-13	Copper-Zinc coated powder
11357-14	Copper-Zinc powder

Tests conducted included irritation studies of the skin and eye, single dose toxicity studies using dermal and oral routes and multiple dose toxicity and sensitization studies using dermal routes. A selective approach to testing was used, in that all test materials were not assayed in all systems. Of the five test materials received, all were assessed for dermal and eye irritancy, and oral toxicity, and three for single dose dermal toxicity. An oral LD50 determination was performed on two of the five materials, a two week multiple dose dermal toxicity and dermal sensitization studies were conducted on another compound. A summary of the test results is noted on the chart following this page.

Inspection of these data show that four of the five test materials were practically non-irritating to the skin of rabbits (Primary Irritation Scores between 0.02 and 0.08) and one sample was non-irritating. Moderate eye irritation occurred from Copper-Zinc coated powder, and mild eye irritation with complete reversibility by day 7 was observed in rabbits treated with Copper-Zinc powder. CI Solvent Yellow 33 and 1-acetyl-3,5,7-trinitro-1,3,5,7-octahydrotriazocine (SEX) were practically non-irritating to the eyes of rabbits, whereas CI Solvent Green 3/CI Solvent Yellow 33 was non-irritating. Copper-Zinc coated powder, showing the highest Draize* scores, caused some residual corneal opacity which resolved by day 14.

The three materials (CI Solvent Green 3/CI Solvent Yellow 33, CI Solvent Yellow 33, and 1-acetyl-3,5,7-trinitro-1,3,5,7-octahydrotriazocine) tested for Dermal Toxicity had LD₅₀ values estimated as greater than 2000 mg/kg. Microscopic examination of treated and untreated skin with CI Solvent Green 3/CI Solvent Yellow 33 and 1-acetyl-3,5,7-trinitro-1,3,5,7-octahydrotriazocine revealed no differences in induced pathology between the two skin sections. CI Solvent Yellow 33 produced minimal to mild hyperkeratosis to treated skin where untreated skin

* Draize, J.H. 1965. Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics. Association of Food and Drug Officials of U.S., Topeka, Kansas, pp. 46-59.

SUMMARY OF FINDINGS
Test Material

Test	BSC Code Number 11357-X				
	8	9	12	13	14
PDI	0.08	0.02	0.0	0.08 ^a	0.06 ^a
PEI	NI	PNI	PNI	Mod.	Mini
ADT	G: 2 g/kg	G: 2 g/kg	G: 2 g/kg		
SDOT	G: 5 g/kg	G: 5 g/kg	G: 5 g/kg	L:5g/kg	L:5g/kg
LD50				G: 3300 mg/kg (Combined male and female)	G: 3300 mg/kg (Male) 2084 mg/kg (Female)
RD-D		Refer to Report			
DCS		Not a Dermal Sensitizer			

LEGEND

- PDI: Primary Dermal Irritation. Values noted are Primary Irritation Scores. Maximum value attainable is 8.0.
- PEI: Primary Eye Irritation. NI means non-irritating, PNI means Practically non-irritating, Mini means minimally irritating and Mod. means moderately irritating.
- ADT: Acute Dermal Toxicity Study in Rabbits. Dose administered was 2 g/kg. Values are noted as LD50 value to dose administered. G: means "greater than".
- SDOT: Single Dose Oral Toxicity in rats. Dose administration was 5 g/kg. Values are noted as LD50 value relative to dose administered. G: means "greater than". L: means "less than".
- LD50: Values noted are based on results of graded doses to multiple groups of animals (oral). G: means "greater than".
- RD-D: Two-Week Multiple Dose Dermal Toxicity. Refer to report for data obtained.

^a Due to the inability to remove the sample from the skin, it was assumed that erythema scores at 24 and 72 hours was 0.

LEGEND (continued):

DCS: Delayed-type Contact Sensitization Test.

Blanks indicate tests that were not conducted. These decisions were made by the sponsor and were based on accumulated toxicity data for each test material.

contained no significant lesions. When screened for oral toxicity, LD₅₀ values were estimated as greater than 5000 mg/kg for three test materials CI Solvent Green 3/CI Solvent Yellow 33, CI Solvent Yellow 33, and 1-acetyl-3,5,7-trinitro-1,3,5,7-octahydrotetrazocine and less than 5000 mg/kg for the remaining two. Copper-zinc coated powder was administered orally to rats at 3300 mg/kg, 1980 mg/kg, and 1188 mg/kg body weight. Only two female rats in the highest dose group died during the study. Thus, the oral LD₅₀ value was estimated as being greater than 3300 mg/kg in male and female (combined) Fischer 344 albino rats. Oral administration of Copper-Zinc powder caused no deaths to the male rats at the highest dose level and thus the oral LD₅₀ value for this sample in male rats was greater than 3300 mg/kg body weight. Deaths did occur in the females resulting in an oral LD₅₀ value of 2084 mg/kg with 95% confidence limits between 1623 mg and 2676 mg/kg body weight. Females were more sensitive than males to both test materials.

CI Solvent Yellow 33 was repeatedly applied to the skin using three graded doses to groups of 5 male and 5 female rabbits. Doses administered were 1000, 200, and 50 mg/kg. These treatments caused no compound related deaths during the study. Upon histopathological examination of treated skin sites, mild to moderate degrees of one or more of the following changes occurred: hyperkeratosis, acanthosis and adnexal hyperplasia. These treated skin sections were compared with untreated skin sections from the same rabbit. There was no definitive increase in severity of these skin lesions with increasing dose levels. All other lesions found on organs were of an incidental nature and it could not be determined from this test whether or not these changes were related to the absorption and toxicity of the test sample.

A Delayed-Type Contact Sensitization study was conducted on CI Solvent Yellow 33. A group of 10 male guinea pigs received a total of 10 topical applications (0.5 grams/application) for 6 hours each, over the course of three weeks. A positive control group of 10 guinea pigs was also employed to insure the validity of the test system. The test sample produced no sensitization (score of 0.0) whereas the positive control sample produced sensitization resulting in a score of 1.45.

INTRODUCTION

The specific objective of the acute studies conducted by Bioassay Systems Corporation (BSC) was to provide toxicity data to the U.S. Army Medical Research and Development Command. Standard measurements of short-term (acute) toxicity include skin and eye irritation studies, dermal and oral toxicity studies, and a dermal sensitization study.

MATERIALS AND METHODS

1. Test Materials

Five powdered test materials were received at Bioassay Systems Corporation. The test materials were stored at room temperature. Aliquots of each material were withdrawn from the bulk material as needed for the various studies. The identification of the materials is as follows:

<u>BSC Code#</u>	<u>Chemical Name</u>
11357-8	CI Solvent Green 3/CI Solvent Yellow 33 (70.9/24.1)
11357-9	CI Solvent Yellow 33*
11357-12	1-acetyl-3,5,7-trinitro-1,3,5,7- octahydrotetrazocine (SEX)
11357-13	Copper-Zinc coated powder**
11357-14	Copper-Zinc powder**

The following information was supplied by the Sponsor.

* Organic Dyes

The dyes tested were:

Yellow Dye - [CI Solvent Yellow No. 33, 2-(2-quinolyl)-1,3-indandione]

Yellow-Green Dye - [a mixture of CI Solvent Yellow No. 33 and CI Solvent Green No. 3 (1,4-di-p-toluidino anthraquinone)]

** Compositions of these compounds are provided on next page.

Chemicals

CI Solvent Yellow No. 33 and the CI Solvent Green No. 3 - CI Solvent Yellow No. 33 mixture were supplied by U.S. Army Medical Bioengineering and Research Development Laboratory (USAMBRDL). Each was analyzed by high pressure liquid chromatography (HPLC; reverse phase column; gradient of 90:10 methanol:water to 100% methanol in 10 minutes; 1 ml/min flow rate; UV detection at 254 nm). CI Solvent Yellow No. 33 was 93.1% 2-(2-quinolyl)-1,3-indandione, 1.8% phthalic acid/anhydride and 0.4% quinaldine by weight. The CI Solvent Green No. 3 - CI Solvent Yellow No. 33 mixture was 24.1% 2-(2'-quinolyl)-1,3-indandione and 70.9% 1,4-di-p-toluidino anthraquinone, 0.6% phthalic acid/anhydride, 0.2% quinaldine, 0.1% O-toluidine and 0.1% quinazarin. Remaining constituents were unknown.

** Composition of Copper-Zinc coated powder and Copper-Zinc Powder

	Copper-Zinc powder (ppm)	Copper-Zinc powder (coated) (ppm)
Ag	20	60
Al	2500-3300	1000-1300
B	95	106
Ba	6	10
Be	0.5	0.18
Ca	20	520
Cd	40	60
Co	2.5	4
Cs	10	20-30
Ga	<150	<150
K	680	830
Mg	7	70
Mn	15-25	20
Na	30	70
Ni	60	120
Si	275	390
Sr	< 0.1	2
Ti	30	30
V	4	4
Zr	5	7
As	3	3
Li	2	3
Fe	< 100-245	2000
Pb	134	105
Hg	.007	.003
Cu	72-69%	70%
Zn	27-20%	25-30%
Se	< 5	< 5
Sb	5	5
Ta	< 0.01	< 0.01
Cl	2%	< 0.2

This information was supplied by the Sponsor

2. Animals

New Zealand white rabbits were used for the irritation and dermal toxicity studies. Fischer 344 albino rats were used for the oral toxicity studies. Male Duncan Hartley guinea pigs were used for the dermal sensitization study.

Rabbits were received from Pine Acres Rabbitry, West Brattleboro, Vermont. These animals were 8-15 weeks old when obtained. The rabbits were individually housed in stainless steel cages, fed Charles River Rabbit Formula (Agway) ad libitum and provided untreated municipal water via water bottles. They were quarantined for two weeks for the dermal and eye irritation and toxicity studies. Fluorescent lighting was controlled to provide a 12-hour light cycle (7 AM to 7 PM).

The rats were received from Taconic Farms, Germantown, New York. These animals were group housed, five per cage, in polycarbonate suspended shoe box type cages. They were fed Charles River R-M-H 3000 feed (Agway) ad libitum except for 16-18 hours prior to dosing at which time food was withdrawn. Water was provided in water bottles on an ad libitum basis. They were quarantined for one week prior to treatment.

The guinea pigs were received from Charles River Breeding Labs, Wilmington, MA. These animals were individually housed in polycarbonate suspended shoe box type cages. They were fed Charles River Guinea Pig Chow (Agway) and water ad libitum. They were quarantined for 2-3 weeks prior to treatment.

The animals were housed in the acute facility. These rooms are provided 12-16 air changes per hour. Animals were maintained at a temperature range of 67°F to 72°F and in a relative humidity range of 35% to 65% as stated in the protocol. However, during several studies the temperature and humidity were out of the range stated in the protocol. These deviations are documented in the raw data. Temperature readings were within $\pm 4^\circ\text{F}$ of the range. Relative humidity deviated from the range by $\pm 11\%$ on a few occasions.

3. Experimental Design

The tests conducted on each of the test materials are noted in the Experimental Design Table. Decisions on which tests to perform were made by the sponsor.

Experimental Design Table

BSC Code *	Primary		Single Dose		Two-Week Multiple Dose	
	Dermal Irritation (0.5g/site)	Primary Eye Irritation (0.1g) ^a	Oral Toxicity (5 g/kg)	Dermal Toxicity (2 g/kg)	Dermal Toxicity (3 graded levels)	Delayed-type Contact Sensitization
11357-8	X	X	X	X		
11357-9	X	X	X	X	X	X
11357-12	X	X	X	X		
11357-13	X	X	X			
11357-14	X	X	X			

Test Methods Noted in Appendices

Appendix	A	A	B	B	C	D	E
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a) 0.1 gram was used for sample 11357-8, 9, 12 and 13. Animals administered sample 11357-14 received 0.08 gram (which equals 0.1 ml).

* See sample names in Materials and Methods Section, 1. Test Materials.

4. Study Elements

Dermal Irritation

A measurement of 0.5 g of the test material was applied to a 1 in. x 1 in. gauze pad, moistened with saline, and secured with surgical tape to two abraded and two unabraded sites per animal. The trunk of the rabbit was covered first with plastic wrap and then with stockinette to prevent disturbance of the test sites.

The test sites were exposed for 24 hours at which time the rabbits were unwrapped and the test sites scored for irritancy using the method of Draize*. The test sites were again scored at 72 hours. In studies where irritation persisted at 72 hours, additional scoring was made for all test sites at day 7. Similarly, irritation on day 7 would trigger further examination at day 14. The last day of scoring, if irritation was present on day 14, was day 21. The specific test methods for the primary dermal irritation study are contained in Appendix A.

Eye Irritation

Animals were selected for inclusion in the primary eye irritation studies based on the absence of any ocular abnormalities. This was ascertained by examination of the eyes with an ophthalmoscope. For each of the powdered test materials assessed for eye irritancy, not more than 100 mg of the test sample was instilled into the everted lower lid of the right eye. The left eye served as the concurrent control. Evaluation of ocular changes were made by the Draize* method at 24, 48 and 72 hours after treatment. Subsequent observations on day 7, 14 and 21 were made if irritation was persistent. The specific test methods for the primary eye irritation study are contained in Appendix A.

Oral Toxicity

Single dose toxicity trials using a dose of 5000 mg/kg were conducted on all five test materials. Graded-dose LD50 studies were subsequently conducted on two of these five materials.

For each of the single dose trials, the test material was administered suspended in a vehicle, corn oil, to each of five male rats weighing 175-250 grams, and each of five female rats, weighing 150-225 grams. Treatment was performed by use of an 18 gauge ball-tipped stainless steel gavage needle attached to a plastic or glass syringe barrel. Animals were observed frequently on the day of dosing and twice daily thereafter for 14 days. Rats dying intercurrently as well as those surviving treatment and sacrificed on day 14 were necropsied. Body weights were measured twice weekly.

Each graded dose LD50 study was preceded by a range finding study using two animals of each sex at each of four dose levels. Three dose levels, selected from the range finding data, were used for the LD50 study. Five males and five females were treated at each dose level.

*Draize, J.H. 1965. Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics. Association of Food and Drug Officials of the U.S., Topeka, Kansas, 46-59.

Observations, body weights and necropsy procedures were similar as for the single dose trials noted above. The specific test methods for conducting the oral toxicity studies are contained in Appendix B.

Dermal Toxicity (Single Dose and Two-Week Multiple Dose)

Single dose toxicity trials using 2000 mg/kg were conducted on three of the test materials. A Two-Week Multiple Dose Toxicity study, using three dose levels, was conducted on one of these test materials.

The hair was carefully removed from the back and sides of each animal prior to testing. Oster animal clippers were used for this procedure. The powdered test material for the single dose trials was applied uniformly to a 6 in. x 6 in. pad at a dose of 2000 mg/kg, moistened with 0.9% sodium chloride, and secured with surgical tape to the previously clipped and abraded test site. The trunk of the animal was wrapped in plastic wrap and then stockinette to prevent removal of the patches by the animal. After a 24 hour exposure period, the wrappings and patches were removed. All animals were observed 3 times on the day of dosing and twice daily thereafter for signs of toxicity. Body weights were determined twice weekly, and necropsies were performed on animals dying intercurrently, as well as those surviving treatment and sacrificed on day 14. Histopathological examination of treated and untreated skin application sites was performed on animals dying during the test period and on 2 animals per sex necropsied at the end of the test period. Test methods for conducting these studies are contained in Appendix C.

In the conduct of the Two-Week Multiple Dose studies, five male and five female rabbits were used for each of three dose levels. Each rabbit was clipped prior to initiation of the study and as needed throughout the test period. All rabbits were treated daily (5 days a week) for two weeks. Animals were wrapped using similar procedures as for the single dose trials. Rabbits remained wrapped for 6 hours each day. Erythema and edema of treated skin sites were evaluated daily for 14 days according to the methods of Draize*. All rabbits were observed at least once daily for signs of toxicity. Body weights and food consumption were measured twice weekly**. A gross necropsy was performed on all animals dying intercurrently as well as those surviving treatment and sacrificed on day 14. Histopathological examination was performed on multiple sections of treated skin, one section of untreated skin, all gross lesions, heart, liver, and kidney for all animals. Test methods for conducting these studies are contained in Appendix D.

Delayed-Type Contact Sensitization Study

A group of 10 male Duncan Hartley guinea pigs received topical applications (0.5 grams/application) of the powdered test sample, as supplied by the Sponsor. The positive control group of 10 male guinea pigs received topical applications (0.5 ml/application) of a 0.1% (w/v) solution of 1-chloro-2,4-dinitrobenzene in 70% ethanol. Each animal received topical applications of the test or positive control sample to a test site on the right side of the animal. The test sample was moistened with Sterile Water prior to application. The

*Draize, pp. 46-59.

**Food consumption was measured on day 3, 7, 10 and 14 of the study.

samples were administered three times weekly (on alternate days) plus one additional day for a total of ten applications. The animals were wrapped for a period of 6 hours for each application. Following the tenth sensitizing treatment, the animals were set aside for two weeks after which each animal then received a challenge topical application (6 hour exposure) of the test or positive control sample on a new (previously unused) test site on the left side of the animal. Erythema, edema and other lesions were scored at 1 and 24 hours after the first application, and the challenge application, according to Draize (1965)*. The animals were weighed on days 1 and 37. An average score was calculated from the first sensitizing treatment and compared to the average score of the challenge treatment. If the value for the challenge reading was substantially higher than for the average of the original readings, the sample can be considered to have produced sensitization. Test method for conducting this study is contained in Appendix E.

5. Histopathology

Designated tissues were harvested at necropsy and fixed in 10% neutral buffered formalin. Microslides were prepared after tissues were infiltrated with paraffin, cut at 5-6 microns and stained with hematoxylin and eosin. Examination of the tissues was performed by Kirby N. Smith, D.V.M.

For each single dose dermal toxicity study, the following tissues were examined:

Treated Skin (2 male and 2 female treated plus animals dying intercurrently)

Untreated Skin (from each animal where treated skin was preserved)

For the two week multiple dose dermal toxicity study, the following tissues were examined for each animal:

Heart
Liver
Kidneys

Treated Skin
Untreated Skin
Gross lesions noted at necropsy

*Draize, pp. 46-59.

RESULTS

Primary Dermal Irritation Test

The summary of primary dermal irritation scores is noted below:

<u>Chemical Name</u>	<u>Primary Irritation Score</u>
CI Solvent Green 3/CI Solvent Yellow 33	0.08
CI Solvent Yellow 33	0.02
SEX	0.0
Copper-Zinc Coated Powder	0.08
Copper-Zinc Powder	0.06

Summaries of these studies follow.

CI Solvent Green 3/CI Solvent Yellow 33

The scoring data are shown in Table 1.

Application of 0.5 g of 11357-8 to 2 abraded and 2 unabraded test sites of 6 rabbits for 24 hours produced barely perceptible erythema to one abraded test site of one male and two female rabbits, and one unabraded site of one male rabbit. This erythema resolved in all cases by 72 hours. No edema was observed at any of the sites during the 24 and 72 hour observation period. The Primary Irritation Score for this test sample was 0.08. Clinical signs indicative of systemic toxicity were not observed.

CI Solvent Yellow 33

The scoring data are shown in Table 2.

Application of 0.5 g of 11357-9 to 2 abraded and 2 unabraded test sites of 6 rabbits for 24 hours produced barely perceptible erythema to one abraded test site of one rabbit. This erythema resolved by 72 hours. There was no edema observed at any of the test sites at the 24 and 72 hour observation periods. The Primary Irritation Score for this test sample was 0.02 (practically non-irritating). Clinical signs indicative of systemic toxicity were not observed.

SEX

The scoring data are shown in Table 3.

Application of 0.5 g of 11357-12 to 2 abraded and 2 unabraded test sites of 6 rabbits for 24 hours produced no erythema or edema at the 24 and 72 hour observation periods. The Primary Irritation Score for the test sample was 0.0 (non-irritating). Clinical signs indicative of toxicity were not observed.

Copper-Zinc coated powder

The scoring data are shown in Table 4.

Application of 0.5 g of 11357-13 to 2 abraded and 2 unabraded test sites of 6 rabbits for 24 hours produced no erythema to the skin of rabbits at the 24 and 72 hour observation periods. At 24 hours, all test sites were covered with the test sample. If erythema was present, it could not be evaluated. By 72 hours, the four test sites of two rabbits were still covered with the sample. Moderate edema was observed at one abraded site, and very slight edema was observed at one unabraded test site of one rabbit after removal of the patches at 24 hours. This edema resolved by the 72 hour observation period. The Primary Irritation Score for this test sample was 0.08 which was based on the assumption that erythema scores at 24 and 72 hours were 0. Clinical signs indicative of toxicity were not observed.

Copper-Zinc Powder

The scoring data are shown in Table 5.

Application of 0.5 g of 11357-14 to 2 abraded and 2 unabraded test sites of 6 rabbits for 24 hours caused very slight edema to the unabraded site and slight edema to the abraded site of one rabbit at 24 hours which resolved by the 72 hour observation period. At 24 hours, the test sites of all 6 rabbits were covered with sample and erythema could not be evaluated. By the 72 hour observation period, only a few test sites were covered with sample and the remaining sites displayed no erythema. The Primary Irritation Score for this test sample was 0.06 which was based on the assumption that erythema scores at 24 and 72 hours were 0. Clinical signs indicative of toxicity were not observed.

LEGEND

Primary Dermal Irritation

Tables 1-5

Observations: E/E - Erythema and Eschar/Edema

Test Site Identity:

RA - Right Anterior
LP - Left Posterior
RP - Right Posterior
LA - Left Anterior

Time Symbols: h - hours
d - days

Sex: M - male
F - female

Mean: Based on 24 and 72 hour readings

Primary Irritation Score: Sum of all erythema/eschar and edema scores for 24 and 72 hours (96 values) divided by the total number of observations (48).

See Appendix A for detailed scoring method.

Maximum Primary Irritation Score = 8.0

TABLE 1^a

PRIMARY DERMAL IRRITATION STUDY

CI Solvent Green 3/CI Solvent Yellow 33

Observations: Erythema and Eschar/Edema

Rabbit Number	Sex	Body Weight(kg)	Abraded				Unabraded				MEAN	
			RA		LP		RP		LA			
			24h E/E	72h E/E	24h E/E	72h E/E	24h E/E	72h E/E	24h E/E	72h E/E		
8502	M	2.6	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0.0
8510	M	2.5	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0.0
8512	M	2.8	0/0	0/0	0/0	1/0	0/0	0/0	0/0	1/0	0/0	0.25
8536	F	2.6	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0.0
8537	F	2.6	0/0	0/0	0/0	1/0	0/0	0/0	0/0	0/0	0/0	0.125
8541	F	2.7	0/0	0/0	0/0	1/0	0/0	0/0	0/0	0/0	0/0	0.125

Primary Irritation Score: 0.08

Rating: Practically non-irritating

^a see legend on page 15

All test sites at 24 and 72 hours were still covered with a slight amount of green sample, however, it was still possible to score for erythema.

TABLE 2^a

PRIMARY DERMAL IRRITATION STUDY

CI Solvent Yellow 33

Observations: Erythema and Eschar/Edema

Rabbit Number	Sex	Body Weights(kg)	Abraded				Unabraded				MEAN	
			RA		LP		RP		LA			
			24h E/E	72h E/E	24h E/E	72h E/E	24h E/E	72h E/E	24h E/E	72h E/E		
8501	M	2.5	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0.0
8514	M	2.7	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0.0
8526	M	2.7	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0.0
8534	F	2.6	0/0	0/0	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0.125
8539	F	2.8	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0.0
8540	F	2.4	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0.0

Primary Irritation Score: 0.02

Rating: Practically non-irritating

^a see legend on page 15

TABLE 3^a

PRIMARY DERMAL IRRITATION STUDY

SEX

Observations: Erythema and Eschar/Edema

Rabbit Number	Sex	Body Weight(kg)	Abraded				Unabraded				MEAN	
			RA		LP		RP		LA			
			24h E/E	72h E/E	24h E/E	72h E/E	24h E/E	72h E/E	24h E/E	72h E/E		
8680	M	2.8	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0.0
8685	M	2.4	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0.0
8699	M	2.3	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0.0
8703	F	2.2	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0.0
8713	F	2.2	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0.0
8715	F	2.6	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0.0

Primary Irritation Score: 0.0

Rating: Non-irritating

^asee legend on page 15

TABLE 4^a

PRIMARY DERMAL IRRITATION STUDY

Copper-Zinc Coated Powder

Observations: Erythema and Eschar/Edema

Rabbit Number	Sex	Body Weights(kg)	Abraded				Unabraded				MEAN
			RA		LP		RP		LA		
			24h E/E	72h E/E	24h E/E	72h E/E	24h E/E	72h E/E	24h E/E	72h E/E	
9461	M	2.5	b/0	b/0	b/0	b/0	b/0	b/0	b/0	b/0	0.0 ^c
9462	M	2.7	b/0	0/0	b/0	0/0	b/0	0/0	b/0	0/0	0.0 ^c
9468	M	2.6	b/0	0/0	b/0	0/0	b/0	0/0	b/0	0/0	0.0 ^c
9480	F	2.4	b/0	b/0	b/0	b/0	b/0	b/0	b/0	b/0	0.0 ^c
9482	F	2.6	b/3	0/0	b/0	0/0	b/0	0/0	b/1	0/0	0.5 ^c
9484	F	2.8	b/0	0/0	b/0	0/0	b/0	0/0	b/0	0/0	0.0 ^c

Primary Irritation Score: 0.08^c

Rating: Practically non-irritating

^a see legend on page 15^b sample could not be removed from test sites so erythema could not be evaluated.^c score based on assumption that erythema scores at 24 and 72 hours were 0.

TABLE 5^a

PRIMARY DERMAL IRRITATION STUDY

Copper-Zinc Powder

Observations: Erythema and Eschar/Edema

Rabbit Number	Sex	Body Weight(kg)	Abraded				Unabraded				MEAN
			RA		LP		RP		LA		
			24h E/E	72h E/E	24h E/E	72h E/E	24h E/E	72h E/E	24h E/E	72h E/E	
9460	M	2.5	b/0	0/0	b/0	0/0	b/0	0/0	b/0	0/0	0.0 ^c
9464	M	2.6	b/2	0/0	b/0	0/0	b/0	0/0	b/1	b/0	0.375 ^c
9466	M	2.6	b/0	b/0	b/0	b/0	b/0	b/0	b/0	b/0	0.0 ^c
9472	F	2.8	b/0	b/0	b/0	b/0	b/0	b/0	b/0	b/0	0.0 ^c
9475	F	2.4	b/0	0/0	b/0	0/0	b/0	0/0	b/0	b/0	0.0 ^c
9478	F	2.4	b/0	b/0	b/0	b/0	b/0	b/0	b/0	b/0	0.0 ^c

Primary Irritation Score: 0.06^c

Rating: Practically non-irritating

^a see legend on page 15^b sample could not be removed from test sites so erythema could not be determined.^c score based on assumption that erythema scores at 24 and 72 hours were 0.

Primary Eye Irritation Studies

The range of total scores observed for the test materials examined by eye irritation studies are noted below. These values are based on readings at 24, 48 and 72 hours.

<u>Chemical Name</u>	<u>Range</u>
CI Solvent Green 3/CI Solvent Yellow 33	0
CI Solvent Yellow 33	0-2
SEX	0-4
Copper-Zinc Coated Powder	0-57
Copper-Zinc Powder	0-30

The maximum score attainable is 110.

Individual study summaries are noted below.

CI Solvent Green 3/CI Solvent Yellow 33

The summary of the ocular scores is noted in Table 6.

Application of 100 mg of the test material to the right eye of 3 rabbits produced no corneal opacity, iritis, hyperemia, chemosis, or discharge throughout the 72 hour observation period. This sample is non-irritating to unirrigated eyes of rabbits. Clinical signs indicative of systemic toxicity were not observed.

CI Solvent Yellow 33

The summary of the ocular scores is noted in Table 7.

Application of 100 mg of the test material to the right eye of 3 rabbits produced no corneal opacity or involvement of the iris throughout the 72 hour observation period. Two of the three eyes displayed hyperemia at 24 hours which resolved in one eye by 48 hours, and in the other eye by 72 hours. No chemosis or discharge were observed during the 72 hour observation period. This sample is minimally irritating to unirrigated eyes of rabbits. Clinical signs indicative of systemic toxicity were not observed during the course of this study.

SEX

The summary of the ocular scores is noted in Table 8.

Application of 100 mg of 11357-12 to the right eye of 3 rabbits produced no corneal opacity or involvement of the iris throughout the 72 hour observation period. Hyperemia and chemosis were observed in the eye of one rabbit 24 hours post-initiation of application. Hyperemia resolved by 72 hours, and chemosis resolved by 48 hours. No discharge from the eyes was observed throughout the course of the study. This sample is minimally irritating to the eyes of rabbits. Clinical signs indicative of systemic toxicity were not observed during the course of this study.

Copper-Zinc Coated Powder

The summary of the ocular scores is noted in Table 9.

An initial eye irritation study was performed and not accepted by the Sponsor due to fluctuations in temperature and humidity and was thus not reported. For the repeat study application of 100 mg of 11357-13 to the right eye of 3 rabbits produced varying degrees of corneal opacity to two of the three treated eyes 24 hours post-initiation of application. The corneal opacity resolved in one eye by day 7 and in the other eye by day 14. Iritis was observed in all three eyes at 24 hours which resolved in one eye by 48 hours, one eye by 72 hours, and in the remaining eye by day 7. Hyperemia, chemosis, and discharge (varying degrees) were observed in all three eyes 24 hours post-initiation of application. Hyperemia resolved in one eye by 72 hours, and in the other two eyes by day 7. Chemosis resolved in one eye by 48 hours, one eye by 72 hours, and in the remaining eye by day 7. Discharge resolved in one eye by 48 hours, and in the other two eyes by 72 hours. This sample is moderately irritating to the eyes of rabbits. One rabbit displayed diarrhea on days 10 through 12 which resolved by day 13. No other signs indicative of systemic toxicity were observed.

Copper-Zinc Powder

The summary of the ocular scores is noted in Table 10.

Application of 80 mg of 11357-14 to the right eye of 3 rabbits produced varying degrees of corneal opacity to two eyes at 24 hours which resolved in one eye by 48 hours and in the other eye by day 7. Only one eye displayed iritis at 24 hours which resolved by the 72 hour observation period. Hyperemia, chemosis and discharge were observed in all three treated eyes 24 hours post-initiation of application. Hyperemia resolved in all three eyes by day 7. Chemosis resolved in all three eyes by 72 hours, and no discharge was observed from the eyes of any rabbits by the 48 hour observation period. This sample is mildly irritating to the eyes of rabbits. Clinical signs indicative of systemic toxicity were not observed during the course of the study.

TABLE 6
PRIMARY EYE IRRITATION STUDY
CI Solvent Green 3/CI Solvent Yellow 33

Rabbit Number	Sex	Body Weights(kg)	Time (hours)	Ocular Scores							Total a Score
				Cornea		Iris	Hyperemia	CONJUNCTIVA		Discharge	
				A	B			Chemosis			
8839	F	2.5	24	0	-	0	0	0	0	0	0
			48	0	-	0	0	0	0	0	0
			72	0	-	0	0	0	0	0	0
8854	F	2.9	24	0	-	0	0	0	0	0	0
			48	0	-	0	0	0	0	0	0
			72	0	-	0	0	0	0	0	0
8859	F	2.7	24	0	-	0	0	0	0	0	0
			48	0	-	0	0	0	0	0	0
			72	0	-	0	0	0	0	0	0

The range of individual scores for the test group was 0.

The average test score for all animals at three observation periods was 0.

^aScoring methods outlined in Appendix A.

TABLE 7
PRIMARY EYE IRRITATION STUDY
CI Solvent Yellow 33

Ocular Scores										
Rabbit Number	Sex	Body Weight(kg)	Time (hours)	Cornea		Iris	CONJUNCTIVA		Discharge	Total ^a Score
				A	B		Hyperemia	Chemosis		
8835	F	2.8	24	0	-	0	1	0	0	2
			48	0	-	0	0	0	0	
			72	0	-	0	0	0	0	
8840	F	2.7	24	0	-	0	0	0	0	0
			48	0	-	0	0	0	0	
			72	0	-	0	0	0	0	
8849	F	2.3	24	0	-	0	1	0	0	2
			48	0	-	0	1	0	0	
			72	0	-	0	0	0	0	

The range of individual scores for the test group was 0 to 2.

The average test score for all animals at three observations periods was 0.7.

^aScoring methods outlined in Appendix A.

TABLE 8
PRIMARY EYE IRRITATION STUDY

Rabbit Number	Sex	Body Weight(kg)	Time (Day)	SEX							Total ^a Score
				Ocular Scores							
				Cornea		CONJUNCTIVA			Discharge		
				A	B	Iris	Hyperemia	Chemosis			
8922	F	2.9	1	0	-	0	0	0	0	0	
			2	0	-	0	0	0	0		
			3	0	-	0	0	0	0		
8847	F	2.6	1	0	-	0	0	0	0	0	
			2	0	-	0	0	0	0		
			3	0	-	0	0	0	0		
8856	F	2.6	1	0	-	0	1	1	0	4	
			2	0	-	0	1	0	0	2	
			3	0	-	0	0	0	0	0	

The range of individual scores for the test group was 0 to 4.

The average test score for all animals at three observation periods was 0.7.

^aScoring methods outlined in Appendix A.

TABLE 9
PRIMARY EYE IRRITATION STUDY
Copper-Zinc Coated Powder

Rabbit Number	Sex	Body Weight(kg)	Time (Day)	Cornea		Ocular Scores				Total ^a Score
				A	B	Iris	Hyperemia	Chemosis	Discharge	
11225	F	2.2	1	0	-	1	3	2	2	19
			2	0	-	0	2	0	0	4
			3	0	-	0	0	0	0	0
			7	0	-	0	0	0	0	0
			14	0	-	0	0	0	0	0
11229	F	2.8	1	2	1	1	3	2	2	29
			2	2	2	1	3	2	1	37
			3	1	1	0	2	0	0	9
			7	0	-	0	0	0	0	0
			14	0	-	0	0	0	0	0
11231	F	2.7	1	2	3	1	3	2	1	47
			2	2	4	1	3	2	1	57
			3	2	4	1	2	1	0	51
			7	1	4	0	0	0	0	20
			14	0	-	0	0	0	0	0

The range of individual scores for the test group was 0 to 57.

The average test score for all three animals, days 1-3 only, was 28.1.

^aScoring methods outlined in Appendix A.

TABLE 10
PRIMARY EYE IRRITATION STUDY
Copper-Zinc Powder

Rabbit Number	Sex	Body Weights(kg)	Time (Day)	Ocular Scores										Total ^a Score ^a
				Cornea		CONJUNCTIVA								
				<u>A</u>	<u>B</u>	<u>Iris</u>	<u>Hyperemia</u>	<u>Chemosis</u>	<u>Discharge</u>					
9623	F	2.2	1	1	0	2	2	0	1	15				
			2	0	0	2	1	0	6					
			3	0	0	1	0	0	2					
			7	0	0	0	0	0	0					
9625	F	2.2	1	0	0	2	2	2	1	10				
			2	0	0	2	1	0	6					
			3	0	0	1	0	0	2					
			7	0	0	0	0	0	0					
9627	F	2.4	1	1	2	2	2	2	1	30				
			2	1	1	2	1	0	16					
			3	1	0	1	0	0	7					
			7	0	0	0	0	0	0					

The range of individual scores for the test group was 0 to 30.

The average test score for all animals, days 1-3 only, was 10.4.

^aScoring methods outlined in Appendix A.

Acute Dermal Toxicity Studies

The summary of acute dermal toxicity ratings is noted below.

<u>Chemical Name</u>	<u>LD50 Rating</u>
CI Solvent Green 3/CI Yellow 33	Greater than 2000 mg/kg
CI Solvent Yellow 33	Greater than 2000 mg/kg
SEX	Greater than 2000 mg/kg

The individual test results are discussed below.

CI Solvent Green 3/CI Solvent Yellow 33

The dose applied on the skin and body weight changes are noted in Table 11.

Application of 2000 mg/kg of 11357-8 to the abraded skin of five male and five female albino rabbits produced no deaths during the 14-day observation period. The only overt sign of toxicity observed during the study was mild diarrhea displayed by female rabbit # 8850 in the afternoon of day 1. This mild diarrhea subsided completely by the morning of day 2. The body weights of 3 male and 4 female rabbits either remained constant or increased during the study. Male rabbit # 8828 lost approximately 100 grams between days 0 and 3, but regained weight by day 7. Female rabbit # 8861 and male rabbit #8810 decreased in weight by 200 grams between days 10 and 14. There were no gross visible lesions detected upon necropsy of all 10 rabbits at the termination of the study.

Pathologic Findings:

<u>Animal No.</u>	<u>Findings</u>
8811 (M)	Treated Skin: No significant lesions Untreated Skin: No significant lesions
8825 (M)	Treated Skin: No significant lesions Untreated Skin: No significant lesions
8836 (F)	Treated Skin: No significant lesions Untreated Skin: No significant lesions
8855 (F)	Treated Skin: No significant lesions Untreated Skin: No significant lesions

There were no lesions caused by the application of CI Solvent Green 3/CI Solvent Yellow 33.

Kirby N. Smith
Kirby N. Smith, DVM
Staff Pathologist

CI Solvent Yellow 33

The dose applied on the skin and body weight changes are noted in Table 12.

Application of 2000 mg/kg of sample 11357-9 to the abraded skin of five male and five female albino rabbits produced no deaths during the 14 day observation period. The only sign of toxicity observed during the study was diarrhea. Mild diarrhea was displayed by female rabbit # 8853 in the afternoon of day 1 which subsided completely by the morning of day 2. Female rabbit # 8838 was observed to have moderate diarrhea prior to sacrifice in the afternoon of day 14. The body weight of female rabbit # 8838 decreased between days 10 and 14. The body weights of 2 male rabbits (# 8816 and # 8826) decreased between days 0 and 3. Male rabbit # 8826 increased in weight by day 7, and rabbit # 8816 regained body weight. The body weights of the remaining 3 male and 4 female rabbits either remained constant or increased during the study.

Upon necropsy, it was observed that 5 male and 2 female rabbits displayed no gross visible lesions. Female rabbit # 8838 had a gaseous cecum and colon with no formed feces in the colon. Female rabbit # 8830 displayed mottled kidneys, and the medial liver lobe of rabbit # 8844 had a small (1 cm x 1 cm) raised white lesion.

Pathologic Findings:

<u>Animal No.</u>	<u>Findings</u>
8816 (M)	Treated Skin: Minimal to mild hyperkeratosis Untreated Skin: No significant lesions
8822 (M)	Treated Skin: Minimal to mild hyperkeratosis Untreated Skin: No significant lesions
8838 (F)	Treated Skin: Minimal to mild hyperkeratosis Untreated Skin: No significant lesions
8852 (F)	Treated Skin: Minimal to mild hyperkeratosis Untreated Skin: No significant lesions

This sample induced an epidermal response characterized by minimal to mild hyperkeratosis. This is presumably due to slightly more rapid keratinocyte metabolism and maturation than normal.

Kirby N. Smith
Kirby N. Smith, DVM
Staff Pathologist

SEX

The dose applied on the skin and body weight changes are noted in Table 13.

Application of 2000 mg/kg of 11357-12 to the abraded skin of five male and five female albino rabbits produced no deaths during the 14-day observation

period. On day 4, female rabbit # 9732 displayed mild diarrhea which resolved by day 5, but was observed again on day 14. This rabbit's body weight increased between days 0 and 3, remained constant between days 3 and 7, but decreased on day 10 and again on day 14. Female rabbit # 9731 was observed to have mild diarrhea during the afternoon of day 13 and on day 14. This rabbit's body weight increased or remained constant between days 0 and 10, but decreased on day 14. Male rabbit # 9723 displayed mild diarrhea on day 6 which resolved by day 7. Male rabbit # 9720 and # 9721 decreased in weight between days 10 and 14. Male rabbit # 9722 decreased in weight between days 0 and 3, regained the weight by day 7, and decreased in weight again by day 14. Female rabbit # 9726 decreased in weight between days 7 and 10 but regained weight by day 14. Male rabbit # 9723 decreased in weight between days 0 and 10 but regained the body weight by day 14. Upon necropsy at terminus, four male and all five female rats displayed no gross visible lesions. Male rabbit # 9722 displayed 3 small white foci on the outer edges of the liver lobes.

Pathologic Findings:

<u>Animal No.</u>	<u>Findings</u>
9716 (M)	Treated Skin: No significant lesions Untreated Skin: No significant lesions
9721 (M)	Treated Skin: No significant lesions Untreated Skin: No significant lesions
9726 (F)	Treated Skin: No significant lesions Untreated Skin: No significant lesions
9728 (F)	Treated Skin: No significant lesions Untreated Skin: No significant lesions

There are no differences in induced pathology of the skin between treated and untreated animals in this study.

For I. A. Mann
Kirby N. Smith, DVM
Staff Pathologist

TABLE 11
ACUTE DERMAL TOXICITY STUDY
CI Solvent Green 3/CI Solvent Yellow 33

<u>Rabbit Number</u>	<u>Sex</u>	<u>Dose (mg/kg)</u>	<u>Total Dose (g)</u>	<u>Body Weight (kg)</u>				
				<u>0</u>	<u>3</u>	<u>Day 7</u>	<u>10</u>	<u>14</u>
8810	M	2000	5.2	2.6	2.6	2.7	2.8	2.6
8811	M	2000	4.8	2.4	2.5	2.6	2.8	2.8
8813	M	2000	5.4	2.7	2.7	2.9	2.9	3.0
8825	M	2000	5.2	2.6	2.7	2.7	2.8	2.9
8828	M	2000	5.6	2.8	2.7	2.8	2.8	3.0
8834	F	2000	5.2	2.6	2.7	2.9	2.9	3.2
8836	F	2000	4.8	2.4	2.5	2.7	2.8	2.8
8850	F	2000	5.8	2.9	2.9	3.0	3.1	3.2
8855	F	2000	5.0	2.5	2.5	2.6	2.6	2.7
8861	F	2000	5.2	2.6	2.6	2.8	2.8	2.6

TOTAL MORTALITY (Deaths/Treated): 0/10

TABLE 12
ACUTE DERMAL TOXICITY STUDY
CI Solvent Yellow 33

<u>Rabbit Number</u>	<u>Sex</u>	<u>Dose (mg/kg)</u>	<u>Total Dose (g)</u>	<u>Body Weight (kg)</u>				
				<u>0</u>	<u>3</u>	<u>Day 7</u>	<u>10</u>	<u>14</u>
8812	M	2000	5.8	2.9	2.9	3.0	3.0	3.1
8816	M	2000	5.2	2.6	2.4	2.6	2.7	2.7
8818	M	2000	5.2	2.6	2.7	2.7	2.7	2.9
8822	M	2000	5.0	2.5	2.5	2.6	2.6	2.8
8826	M	2000	5.4	2.7	2.6	2.8	2.9	3.0
8830	F	2000	5.6	2.8	2.8	3.0	3.1	3.2
8838	F	2000	5.0	2.5	2.5	2.7	2.7	2.4
8844	F	2000	5.4	2.7	2.7	2.8	2.9	3.1
8852	F	2000	5.2	2.6	2.6	2.8	2.8	3.0
8853	F	2000	4.8	2.4	2.4	2.6	2.7	2.8

TOTAL MORTALITY (Deaths/Treated): 0/10

TABLE 13
ACUTE DERMAL TOXICITY STUDY
SEX

<u>Rabbit Number</u>	<u>Sex</u>	<u>Dose (mg/kg)</u>	<u>Total Dose (g)</u>	<u>Body Weight (kg)</u>				
				<u>Day</u>				
				<u>0</u>	<u>3</u>	<u>7</u>	<u>10</u>	<u>14</u>
9716	M	2000	4.4	2.2	2.2	2.4	2.4	2.4
9720	M	2000	4.6	2.3	2.4	2.5	2.7	2.5
9721	M	2000	4.8	2.4	2.5	2.7	2.9	2.6
9722	M	2000	4.4	2.2	2.1	2.2	2.2	2.1
9723	M	2000	4.6	2.3	2.2	2.1	2.1	2.3
9726	F	2000	4.2	2.1	2.3	2.4	2.3	2.4
9728	F	2000	4.2	2.1	2.2	2.3	2.4	2.5
9729	F	2000	4.4	2.2	2.2	2.3	2.4	2.4
9731	F	2000	4.2	2.1	2.1	2.2	2.2	2.1
9732	F	2000	4.2	2.1	2.3	2.3	2.1	1.8

TOTAL MORTALITY (Deaths/Treated): 0/10

Single Dose Oral Toxicity Studies

The summary of the LD50 ratings for the test materials is noted below.

<u>Chemical Name</u>	<u>LD50 Rating</u>
CI Solvent Green 3/CI Yellow 33	Greater than 5000 mg/kg
CI Solvent Yellow 33	Greater than 5000 mg/kg
SEX	Greater than 5000 mg/kg
Copper-Zinc Coated Powder	Less than 5000 mg/kg
Copper-Zinc Powder	Less than 5000 mg/kg

Summaries of these studies follow.

CI Solvent Green 3/CI Solvent Yellow 33

The dose volume administered, mortality, and body weight change are noted in Table 14.

A single oral administration of 11357-8 in corn oil to five male and five female F344 albino rats at a dose of 5000 mg/kg caused no deaths to the rats throughout the 14 day observation period. All five male and four of the female rats gained or maintained weight throughout the course of the study. Female rat # 11424 lost weight between days 7 and 11 but gained weight by day 14. By day 2 of the observation period, the fur on all 10 rats appeared green in color. On day 4, the fur and tail of the male rats were green, and the fur and tail of the female rats were pale green. By day 14, the male rats were light green in color. The females appeared light greenish/yellow by day 5, and yellow by day 9. By day 8, the males were light green in color. Upon necropsy at terminus, there were no gross internal visible lesions detected in any of the rats. However, all five females were yellow in color, and all five male rats were light green in color.

CI Solvent Yellow 33

The dose volume administered, mortality, and body weight change are noted in Table 15.

A single oral administration of 11357-9 in corn oil to five male and five female F344 albino rats at a dose of 5000 mg/kg caused one compound-related death to one male rat (# 11399) by day 5. Upon necropsy, the stomach was small and contained a yellow granular solid, the intestines contained a small amount of yellow gel, and the cecum was impacted with green solid material. Male rat # 11397 appeared thin on day 6, and thin and dehydrated on day 7. By day 8, this rat displayed mild lethargy, hunched posture, and piloerection. This rat died in the afternoon on day 8. One male and one female rat were found dead during the study due to gavage errors. All surviving rats gained or maintained weight throughout the course of the study. Female rat # 11441 displayed mild diarrhea on the day of dosing which subsided completely by day 1. By day 2, the fur of all ten rats appeared yellow in color. On day 4, the fur and tail of all rats were yellow. Of the seven rats surviving until day 14, yellow liquid was observed in the intestines of three females at necropsy. The remaining rats displayed no internal gross visible lesions. The three male rats were light green in color, and the female rats appeared yellow.

SEX

The dose volume administered, mortality, and body weight change are noted in Table 16.

A single oral administration of 11357-12 in corn oil to five male and five female F344 albino rats at a dose of 5000 mg/kg caused no deaths to the rats. All rats gained or maintained weight throughout the study, and displayed no signs of toxicity. Upon necropsy at terminus, four female and three male rats were observed to have yellow liquid in the intestines. The remaining two male and one female rats displayed no gross visible lesions.

Copper-Zinc Coated Powder

The dose volume administered, mortality, and body weight change are noted in Table 17.

A single oral administration of 11357-13 in corn oil to five male and five female F344 albino rats at a dose of 5000 mg/kg produced compound related deaths to three male and four female rats. One female and two male rats died due to gavage errors. The most commonly displayed signs of toxicity exhibited by the three male and four female rats which died due to the compound included abnormal excreta (gold colored diarrhea), lethargy, hunched posture, and piloerection. Other signs of toxicity included lacrimation (clear or red), and ataxia. Upon necropsy, the anal area of two male rats had greenish-black dried diarrhea. The gastrointestinal tract of the rats in most cases was filled with gold and/or green colored liquid. The stomach of the two male rats found dead on days 5 and 8 was distended and contained the sample. The intestines of both animals were darkened (black or brown) and contained dark colored liquid. The cecum of one of these rats contained green gel, and the cecum of the other rat appeared black and contained green mucoid material.

Copper-Zinc Powder

The dose volume administered, mortality, and body weight change are noted in Table 18.

A single oral administration of 11357-14 in corn oil to five male and five female F344 albino rats at a dose of 5000 mg/kg caused compound related death to four male and five female rats. One male rat died due to a gavage error. The most commonly displayed signs of toxicity exhibited by these rats included abnormal excreta (gold diarrhea), lethargy, hunched posture, and piloerection. Other signs of toxicity included ataxia, lacrimation (clear), and squinted eyes. The tail of male rat #11408 appeared blue-black in color on day 7, and blue-black, thin and dehydrated on day 8 prior to being found dead on day 9. Upon necropsy of the rats found dead during the study, the anal area was stained with diarrhea. Parts or all of the gastrointestinal tract of most of the rats contained gold and/or green liquid.

TABLE 14
SINGLE DOSE ORAL TOXICITY STUDY
CI Solvent Green 3/CI Solvent Yellow 33

Rat Number	Sex	Dose (mg/kg)	Total Dose (ml) ^a	Body Weight (g)					Day of Sacrifice
				0	4	Day 7	11	14	
11381	M	5000	3.9	196	209	228	242	252	14
11376	M	5000	3.8	192	206	229	234	242	14
11404	M	5000	4.1	207	216	235	246	255	14
11392	M	5000	3.8	189	200	220	232	238	14
11401	M	5000	3.8	192	207	221	242	242	14
11424	F	5000	3.2	161	171	177	176	182	14
11431	F	5000	3.3	167	174	182	184	188	14
11432	F	5000	3.3	163	169	176	183	184	14
11437	F	5000	3.5	174	177	186	191	194	14
11442	F	5000	3.2	160	167	175	175	179	14

TOTAL MORTALITY (Deaths/Treated): 0/10

^aBased on a concentration of the sample in corn oil at 0.25 g/ml.

TABLE 15
SINGLE DOSE ORAL TOXICITY STUDY
CI Solvent Yellow 33

Rat Number	Sex	Dose (mg/kg)	Dose Volume (ml) ^a	Body Weight (g)					Day of Death or sacrifice
				0	4	Day 7	11	14	
11387	M	5000	3.9	194	206	225	237	247	14
11390	M	5000	4.0	202	209	232	247	255	14
11391	M	5000	3.9	196	203	223	233	242	14
11397	M	5000	3.9	195	167	156	b	b	8
11399	M	5000	3.8	192	204	c	c	c	5
11419	F	5000	3.3	166	175	187	193	193	14
11426	F	5000	3.2	162	146	135	122	b	13
11433	F	5000	3.4	168	176	188	193	196	14
11436	F	5000	3.1	153	159	169	169	173	14
11441	F	5000	3.3	167	171	183	187	187	14

TOTAL MORTALITY (Deaths/Treated): 1/8^d

^aBased on a concentration of the sample in corn oil at 0.25 g/ml.

^bAnimal died due to gavage error.

^cAnimal died due to toxicity of compound.

^dTwo animals died due to gavage error.

TABLE 16
SINGLE DOSE ORAL TOXICITY STUDY
SEX

Rat Number	Sex	Dose (mg/kg)	Dose Volume (ml) ^a	Body Weight (g)					Day of Sacrifice
				0	4	Day 7	11	14	
11378	M	5000	3.9	194	202	222	233	240	14
11384	M	5000	3.8	190	196	221	234	242	14
11395	M	5000	3.8	192	204	224	237	242	14
11394	M	5000	4.0	202	211	230	244	247	14
11405	M	5000	3.9	197	208	229	243	249	14
11418	F	5000	3.4	172	173	182	183	185	14
11421	F	5000	3.2	159	167	172	172	178	14
11427	F	5000	3.3	166	170	180	184	187	14
11430	F	5000	3.2	162	166	176	180	184	14
11440	F	5000	3.3	164	168	177	181	183	14

TOTAL MORTALITY (Deaths/Treated): 0/10

^aBased on a concentration of the sample in corn oil at 0.25 g/ml.

TABLE 17
SINGLE DOSE ORAL TOXICITY STUDY
Copper-Zinc Coated Powder

Rat Number	Sex	Dose (mg/kg)	Dose Volume (ml) ^a	Body Weight (g)					Day of Death
				0	4	Day 7	11	14	
11377	M	5000	4.0	198	146	c	c	c	5
11379	M	5000	4.1	206	b	b	b	b	1
11393	M	5000	3.9	194	b	b	b	b	1
11410	M	5000	3.9	197	c	c	c	c	3
11406	M	5000	3.7	186	141	122	c	c	8
11425	F	5000	3.4	168	b	b	b	b	1
11429	F	5000	3.2	159	c	c	c	c	2
11435	F	5000	3.2	161	c	c	c	c	1
11438	F	5000	3.3	163	c	c	c	c	3
11444	F	5000	3.3	167	c	c	c	c	1

TOTAL MORTALITY (Deaths/Treated): 7/7^d

^aBased on a concentration of the sample in corn oil at 0.25 g/ml.

^bAnimal died due to gavage error.

^cAnimal died due to compound.

^dThree animals died due to gavage errors.

TABLE 18
SINGLE DOSE ORAL TOXICITY STUDY
Copper-Zinc Powder

Rat Number	Sex	Dose (mg/kg)	Dose Volume (ml) ^a	Body Weight (g)					Day of Death
				0	4	Day 7	11	14	
11380	M	5000	3.9	194	b	b	b	b	2
11385	M	5000	3.9	193	c	c	c	c	1
11398	M	5000	3.8	191	144	b	b	b	6
11408	M	5000	4.0	201	157	133	b	b	9
11409	M	5000	4.2	211	162	b	b	b	5
11423	F	5000	3.2	162	b	b	b	b	4
11417	F	5000	3.3	164	b	b	b	b	1
11420	F	5000	3.2	162	b	b	b	b	2
11434	F	5000	3.1	155	b	b	b	b	1
11443	F	5000	3.2	161	b	b	b	b	2

TOTAL MORTALITY (Deaths/Treated): 9/9^d

^aBased on a concentration of the sample in Corn oil at 0.25 g/ml

^bAnimal died due to compound.

^cAnimal died due to gavage error.

^dOne animal died due to gavage error.

Acute Oral LD50 Determination

The summary of LD50 determinations is noted below.

<u>Chemical Name</u>	<u>Sex</u>	<u>LD50 (mg/kg)</u>	<u>95% Confidence Limits (mg/kg)</u>
Copper-Zinc Coated Powder	Male & Female (combined)	Greater than 3300	Not applicable
Copper-Zinc Powder	Male	Greater than 3300	Not applicable
	Female	2084	1623-2676

The individual studies are summarized as follows.

Copper-Zinc Coated Powder

The results of the range finding study and LD50 determination (Finney*, 1952) are noted in Table 19.

For the acute oral range finding determination two male and two female rats were treated at four different dose levels. An acute oral LD50 determination was then performed on five male and five female rats at each of three dose groups. The rats were administered doses of 3300 mg, 1980 mg or 1188 mg/kg body weight of the sample in corn oil. This sample caused death to two female rats receiving the test sample at a dose of 3300 mg/kg by day 6 of the observation period. Between days 0 and 3, all five male and the four surviving female rats decreased in body weight. The five male rats began to increase in weight by day 7 and throughout the remainder of the study. Between days 3 and 7, two of the three surviving female rats decreased in weight, and one began to increase in weight. Between days 7 and 10, two rats increased in weight and the body weight of the remaining rat decreased. By day 14, body weights of all three rats increased. The most commonly displayed signs of toxicity exhibited by these rats included diarrhea, hunched posture, and piloerection. Others signs of toxicity exhibited by rats in this group included lethargy, squinted eyes, epistaxis, and polypnea. All signs of toxicity exhibited by the male rats resolved by day 11. All signs of toxicity exhibited by the female rats resolved by day 13. The two rats necropsied during the study displayed dark green gel from the stomach to the anus. The anal area of one rat was stained green. The stomach of one rat contained oily brown-gold mash. All rats necropsied at terminus displayed no gross visible lesions.

All five male and five female rats receiving the test sample at a dose of 1980 mg/kg survived throughout the 14-day observation period. Five male and four of the five female rats decreased in weight between days 0 and 3. The body weight of the one other female rat remained constant between days 0 and 3. All ten rats increased in weight by day 7 and throughout the remainder of the study. The most commonly displayed signs of toxicity exhibited by these rats included diarrhea and hunched posture. Other signs of toxicity exhibited by these rats included lethargy, piloerection, and red lacrimation. All signs of toxicity exhibited by these rats resolved by day 6. All ten rats necropsied at terminus displayed no gross visible lesions.

*Finney, D.J., 1952. LD₅₀ value determined using Karber's Method (revised). In Probit Analysis, 2nd Edition. University Press, Cambridge.

The five male and five female rats receiving the test sample at a dose of 1188 mg/kg survived until day 14 of the observation period. Four male and four female rats decreased in weight between days 0 and 3 but increased in weight by day 7 and throughout the remainder of the study. The remaining male rat gained weight between day 0 and 14, and the weight of the remaining female rat remained constant between days 0 and 3 but increased during the 14 day study. The most commonly displayed sign of toxicity exhibited by these rats was diarrhea. Other signs observed included hunched posture, lethargy, and piloerection. All signs of toxicity resolved by day 5. There were no gross visible lesions detected upon necropsy of the ten rats at terminus.

Conclusion:

Due to insufficient mortality rates, an oral LD₅₀ value could not be determined. The estimated oral LD₅₀ value for Copper-Zinc Coated Powder was greater than 3300 mg/kg body weight in male and female (combined) Fischer 344 albino rats.

TABLE 19

ACUTE ORAL LD50 DETERMINATION IN F344 RATS

Copper-Zinc Coated Powder

Dose (mg/kg)	Average Volume Administered (ml) (a)	Range Finding												Total Mortality Deaths/Treated
		Mean Body Weight (g)			Deaths									
		Days After Treatment			Days After Treatment			Days After Treatment		Days After Treatment				
		0	7	14	0-2	3	4	5	6	7	8-14			
<u>Males</u>														
3500	4.9		209	-	-	-	-	1	-	1	-	2/2		
1050	1.5		207	219	249		-	-	-	-	-	0/2		
315	0.42		199	218	232		-	-	-	-	-	0/2		
94.5	0.14		210	230	247		-	-	-	-	-	0/2		
<u>Females</u>														
3500	3.8		164	---	---		-	2	-	-	-	2/2		
1050	1.2		166	176	186		-	-	-	-	-	0/2		
315	0.35		163	180	184		-	-	-	-	-	0/2		
94.5	0.11		161	175	183		-	-	-	-	-	0/2		

(a) Based on a concentration of the test sample in vehicle at 0.15 g/ml.

TABLE 19 (Continued)
ACUTE ORAL LD50 DETERMINATION IN F344 RATS
Copper-Zinc Coated Powder

<u>LD50 Determination</u>																
Dose (mg/kg)	Average Volume Administered (ml) (a)	Mean Body Weight (g)					Deaths							Total Mortality Deaths/Treated		
		Days After Treatment					Days After Treatment									
		0	3	7	10	14	0	1	2	3	4	5	6		7-14	
<u>Males</u>																
3300	3.5	210	181	194	210	236	-	-	-	-	-	-	-	-	-	0/5
1980	2.1	212	187	210	228	249	-	-	-	-	-	-	-	-	-	0/5
1188	1.3	210	201	226	237	252	-	-	-	-	-	-	-	-	-	0/5
<u>Females</u>																
3300	2.6	160	140	123	132	160	-	1	-	-	-	-	-	1	-	2/5
1980	1.6	159	147	161	168	180	-	-	-	-	-	-	-	-	-	0/5
1188	0.94	159	157	168	172	179	-	-	-	-	-	-	-	-	-	0/5

(a) Based on a concentration of the test sample in the vehicle at a concentration of 0.2 g/ml.

Copper-Zinc Powder

The results of the range finding study and LD50 determination (Finney*, 1952) are noted in Table 20. The dose response graph for female rats is noted in Figure 1.

The acute oral range finding determination on 11357-14 utilized two male and two female rats at 4 different dose levels. Based on the results of this test, an acute oral LD50 determination was performed on five male and five female F344 rats at 3 different groups. The rats were administered doses of 3300, 1980, and 1188 mg/kg body weight of the sample in corn oil. Sample 11357-14 caused death to all five female rats receiving the test sample at a dose of 3300 mg/kg by day 7 of the observation period. The five male rats decreased in weight between days 0 and 7 but increased in weight by days 10 and 14. The one other male rat decreased in weight between days 0 and 10 but increased in weight by day 14. The most commonly displayed signs of toxicity exhibited by these rats included diarrhea, lethargy, hunched posture, and piloerection. Other signs of toxicity included epistaxis and tetanus. All signs of toxicity exhibited by the male rats resolved by day 14. The stomach of the five female rats necropsied during the study contained a gold paste. Parts or all of the gastrointestinal tract (stomach to anus) contained dark green gel. All five male rats necropsied at terminus displayed no gross visible lesions.

Two female rats receiving the test sample at a dose of 1980 mg/kg were found dead by day 7 of the observation period. The five male rats decreased in body weight between days 0 and 3, but increased in weight by days 10 and 14. The most commonly displayed signs of toxicity exhibited by these rats included diarrhea, hunched posture, piloerection, and lethargy. Other signs of toxicity included squinted eyes, ataxia, and wheezing. All signs of toxicity exhibited by the surviving rats resolved by day 8. One female rat necropsied during the study was partially cannibalized. The stomach of this rat contained gold paste and gas. Dark green gel was observed in the gastrointestinal tract between the stomach and anus. The stomach of the other rat contained dark green paste and the intestinal tract contained dark green gel. All rats necropsied at terminus displayed no gross visible lesions.

The ten rats receiving the test sample at a dose of 1188 mg/kg survived throughout the 14 day observation period. Five male and four of the five female rats decreased in weight between days 0 and 3 but increased in weight by day 7 and throughout the remainder of the study. The body weight of the remaining female rat remained constant between days 0 and 3 but increased throughout the course of the study. The most commonly displayed signs of toxicity exhibited by these rats included diarrhea and hunched posture. Other signs observed included red lacrimation, lethargy and piloerection. All signs of toxicity resolved in the female rats by day 3, and in the male rats by day 5. All rats necropsied at terminus displayed no gross visible lesions.

* Finney, 1952.

CONCLUSION:

Copper-Zinc Powder has an oral LD₅₀ value of greater than 3300 mg/kg body weight in male Fischer 344 albino rats. The LD₅₀ value for the female rats is 2084 mg/kg with 95% confidence limits between 1623 mg and 2676 mg/kg body weight.

TABLE 20

ACUTE ORAL LD50 DETERMINATION IN F344 RATS
(Copper-Zinc Powder)

Range Finding

Dose (mg/kg)	Average Volume Administered (ml) (a)	Mean Body Weight (g)		Deaths							Total Mortality Deaths/Treated	
		Days After Treatment		0	Days After Treatment			7-14				
<u>Males</u>												
3500	4.6	194	-	-	1	-	-	-	-	1	-	2/2
1050	1.4	202	202	238	-	-	-	-	-	-	-	0/2
315	0.41	196	217	231	-	-	-	-	-	-	-	0/2
94.5	0.13	199	222	230	-	-	-	-	-	-	-	0/2
<u>Females</u>												
3500	3.8	161	-	-	-	1	-	-	-	-	-	2/2
1050	1.1	159	165	177	-	-	-	-	-	-	-	0/2
315	0.34	161	171	181	-	-	-	-	-	-	-	0/2
94.5	0.10	160	176	179	-	-	-	-	-	-	-	0/2

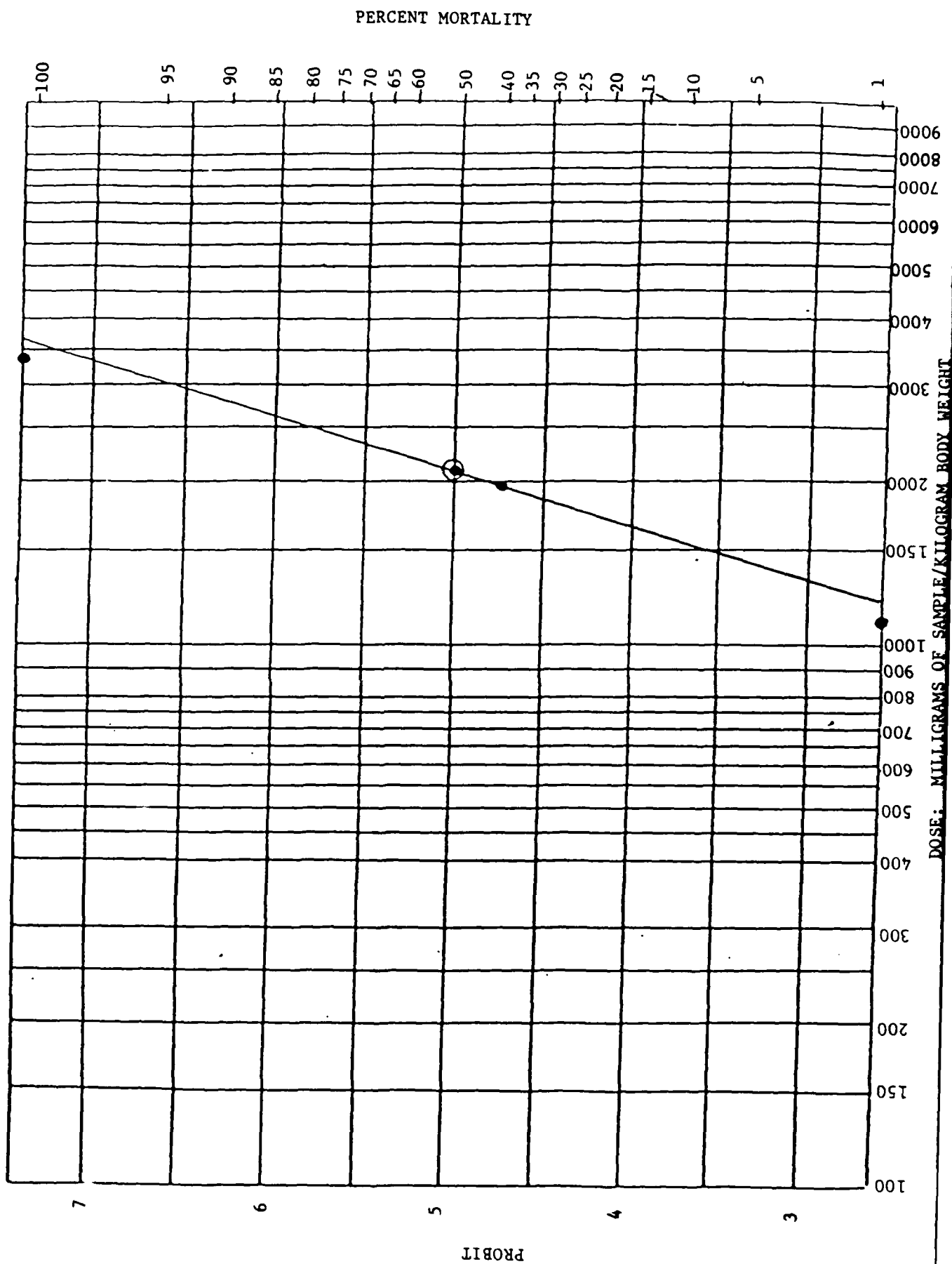
(a) Based on a concentration of the test sample in the vehicle at 0.15 g/ml.

TABLE 20 (Continued)
ACUTE ORAL LD50 DETERMINATION IN F344 RATS
(Copper-Zinc Powder)

<u>LD50 Determination</u>														
Dose (mg/kg)	Average Volume Administered (ml)	Mean Body Weight (g)				Deaths						Total Mortality Deaths/Treated		
		Days After Treatment				Days After Treatment								
		0	3	7	10	14	0-2	3	4	5	6	7	8-14	
<u>Males</u>														
3300	3.4	206	177	144	166	199	-	-	-	-	-	-	-	0/5
1980	2.1	209	182	199	216	240	-	-	-	-	-	-	-	0/5
1188	1.3	210	192	216	230	250	-	-	-	-	-	-	-	0/5
<u>Females</u>														
3300	2.6	159	137	-	-	-	-	1	1	2	-	1	-	5/5
1980	1.6	161	143	130	151	168	-	1	-	-	-	1	-	2/5
1188	0.94	159	154	166	172	179	-	-	-	-	-	-	-	0/5

(a) Based on a concentration of the test sample in vehicle at 0.2 g/ml.

FIGURE 1 - DOSE RESPONSE CURVE FOR COPPER-ZINC POWDER FOR FEMALE F344 ALBINO RATS
ACUTE ORAL LD₅₀ DETERMINATION*



*Finney, 1952

Two Week Multiple Dose Dermal Toxicity Study

CI Solvent Yellow 33

The body weight data, food consumption (measured on day 3, 7, 10 and 14) data and clinical signs of the animals treated with sample number 11357-9 are noted in Tables 21-24.

An initial Two Week Multiple Dose Dermal Toxicity study was performed and terminated 4 days later due to fluctuations in temperature and humidity. For the repeat study, the dose levels administered were 1000, 200, and 50 mg/kg body weights. All rabbits receiving repeated applications of the test sample at 1000 mg/kg survived throughout the entire study. Two female rabbits (#11233 and 11227) decreased in weight between days 0 and 3. Female rabbit #11233 increased in weight by day 7 and maintained or gained weight during the remainder of the study. The body weight of female rabbit #11227 remained constant between days 3 and 7, increased by day 10, and decreased again on day 14. Male rabbit # 11221 decreased in weight between days 7 and 10 and maintained weight between days 10 and 14. Male rabbit # 11207 decreased in weight between days 7 and 10 but regained the weight by day 14. All other rabbits either maintained or gained weight during the study. Scattered observations of mild diarrhea and nasal discharge were the only other signs of toxicity exhibited by these rabbits during the 14 day observation period. In most cases, there was no erythema observed at the treated sites of these rabbits during the 14 day observation period. Where erythema occurred, it was evaluated as barely perceptible (grade 1). There was no edema observed at any of the skin sites throughout the study. Upon necropsy at terminus, four male and four female rabbits displayed no gross visible lesions. The one male rabbit was observed to have scattered 1 mm X 1 mm white lesions on the lobes of the liver. Petechial hemorrhages were observed on both kidneys of the remaining female rabbit.

The five male and five female rabbits receiving the test sample at a dose of 200 mg/kg body weight survived throughout the entire 14 day observation period. Four male and three female rabbits maintained or increased body weight during the 14 day study. One male and one female rabbit decreased in weight between days 0 and 3 but increased in weight by day 7. The other female rabbit decreased in weight between days 7 and 10 but increased in weight by day 14. There were scattered observations of mild nasal discharge and mild to moderate diarrhea during the course of the study. In most cases, there was no erythema observed at the treated sites of these rabbits during the 14 day observation period. Where erythema occurred, it was evaluated as barely perceptible (grade 1). There was no edema observed at any of the skin sites throughout the study. Upon necropsy at terminus, two male and all five female rabbits displayed no gross visible lesions. The medial liver lobe of one male rabbit displayed a circular pattern with 2 mm X 2 mm white circular lesions. The colon of another male rabbit was gaseous, and the large intestine of the remaining male rabbit was gaseous and contained watery diarrhea. Necropsy findings at 1000, 200 and 50 mg/kg dose levels are provided in Tables 25, 26 and 27, respectively.

Three male and all five female rabbits receiving the test sample at a dose of 50 mg/kg survived until the termination of the study. Male rabbit #11223 was found dead on day 2, trapped between the bars of the cage. This rabbit most probably struggled and kicked sufficiently to break its back. The other male rabbit (#11212) found dead on day 10 displayed multifocal suffusion

hemorrhages in the duodenum, mucus in the colon, and a dark walled cecum. This rabbit decreased significantly in body weight between days 3 and 7, and days 7 and death (day 10). This rabbit's food intake decreased significantly during these two time periods. One male (#11215) and one female (#11236) rabbit lost weight between days 0 and 3. Three male and four female rabbits maintained or gained weight between days 0 and 3. By day 7, these two rabbits (# 11215 and 11236) increased in weight. The body weight and food intake of female rabbit #11236 decreased between days 7 and 10 but increased again by day 14. Only one male rabbit (#11216) decreased in weight between days 10 and 14. One male rabbit (#11218) displayed mild diarrhea on day 14. In most cases, there was no erythema observed at the treated sites of these rabbits during the 14 day observation period. Where erythema occurred, it was evaluated as barely perceptible (grade 1). There was no edema observed at any of the skin sites throughout the study. Upon necropsy at terminus, two female and two male rabbits displayed no gross visible lesions. Both kidneys of two female rabbits displayed scattered beige areas, and both kidneys of one of these rabbits displayed petechial hemorrhages. The colon of one male rabbit appeared gaseous, and the lobes of the liver of one female rabbit displayed scattered white circular lesions, 1 mm X 1 mm in size.

Pathologic Findings:

<u>Dose (mg/kg)</u>	<u>Animal Numbers</u>	<u>Organ</u>	<u>Pathology</u>
50	Males: 11215, 11223*, 11216, 11218, 11212*	Skin:	Untreated: No significant lesions (all). Treated: Moderate hyperkeratosis (11216, 11215, 11218, 11212). No significant lesions (11223). Injured Area (11223)**: Diffuse subcutaneous hemorrhages.
		Heart:	Multifocal chronic inflammation moderate (11215), mild (11216, 11212). No significant lesions (others).
		Liver:	Multifocal chronic cholangiohepatitis, moderate (11212, 11218), marked (11215), mild (11216). No significant lesions (11223).
		Kidneys:	Multifocal chronic interstitial nephritis, moderate (11215, 11216), marked (11212).

* Animals died before study completion.

** Skin injured due to accident, not chemical.

<u>Dose (mg/kg)</u>	<u>Animal Numbers</u>	<u>Organ</u>	<u>Pathology</u>
50	Males (continued)	Intestines: (11212)	Duodenum: Diffuse congestion; multifocal hemorrhages. Colon: Mucus cast. Cecum: Diffuse congestion. Multifocal tubular vacuolation, and focus of mineralization (11218). Marked diffuse tubular dilation, degeneration, and casts (11212). No significant lesions (11223).

<u>Dose (mg/kg)</u>	<u>Animal Numbers</u>	<u>Organ</u>	<u>Pathology</u>
50	Females: 11232, 11236, 11243, 11238, 11241	Skin:	Untreated: No significant lesions (all). Treated: Mild hyperkeratosis (11243). Hyperkeratosis, acanthosis and adnexal hyperplasia, mild (11238), moderate (11241), marked (11232, 11236).
		Heart:	Multifocal chronic inflammation, minimal (11232, 11241), mild (11236), moderate (11238). No significant lesions (11243).
		Liver:	Multifocal proliferative parasitic chronic cholangiohepatitis (11232). Focal necrosis with chronic multifocal mononuclear inflammation (11236). Diffuse moderate fatty change (11243). Mild diffuse chronic pericholangitis (11238, 11241).
		Kidney:	Multifocal chronic mononuclear interstitial nephritis, moderate (11232, 11236, 11241); marked with tubular dilation, degeneration and casts (11238). No significant lesions (11243).

<u>Dose (mg/kg)</u>	<u>Animal Numbers</u>	<u>Organ</u>	<u>Pathology</u>
200	Males: 11204, 11210, 11214, 11205, 11208	Skin:	Untreated: No significant lesions (all). Treated: Mild hyperkeratosis (11204, 11214, 11210). Moderate hyperkeratosis, (11205). Moderate hyperkeratosis and acanthosis (11208).
		Heart:	No significant lesions.
		Liver:	Diffuse fatty change, marked (11204), moderate (11205), mild (11210). Chronic pericholangitis, mild (11214). Severe multifocal necrotizing chronic granulomatous hepatitis (11205). No significant lesions (11208).
		Kidney:	Moderate multifocal chronic interstitial nephritis (11214, 11205). No significant lesions (others).

<u>Dose (mg/kg)</u>	<u>Animal Numbers</u>	<u>Organ</u>	<u>Pathology</u>
200	Females: 11224, 11228, 11242, 11234, 11239	Skin:	Untreated: No significant lesions (all). Treated: Hyperkeratosis and acanthosis, mild (11224, 11228, 11242, 11239); moderate with adnexal hyperplasia (11234).
		Heart:	Multifocal mononuclear inflammation (11234, 11239). No significant lesions (others).
		Liver:	Focal necrosis (11242). Multifocal chronic mononuclear inflammation, mild (11234, 11239). Fatty change, diffuse, mild (11242, 11239), moderate (11234). No significant lesions (11224, 11228).
		Kidney:	Multifocal chronic mononuclear interstitial nephritis, mild (11224), moderate (11234). No significant lesions (11228, 11242, 11239).

<u>Dose (mg/kg)</u>	<u>Animal Numbers</u>	<u>Organ</u>	<u>Pathology</u>
1000	Males: 11213, 11222, 11221, 11219, 11207	Skin:	Untreated: No significant lesions (all). Treated: Moderate hyperkeratosis and acanthosis, with mild adnexal hyperplasia (all).
		Heart:	Minimal focal chronic inflammation (11221). No significant lesions (others).
		Liver:	Diffuse fatty change, marked (11213), moderate (11207), mild (11219, 11222). Severe focal chronic proliferative parasitic cholangitis (11207). No significant lesions (11221).
		Kidney:	Focal moderate chronic interstitial nephritis with tubular degeneration and dilation (11221). No significant lesions (11213, 11222, 11219, 11207).

<u>Dose (mg/kg)</u>	<u>Animal Numbers</u>	<u>Organ</u>	<u>Pathology</u>
1000	Females: 11230, 11237, 11233, 11240, 11227	Skin:	Untreated: No significant lesions (all). Treated: Hyperkeratosis and acanthosis, moderate, with mild adnexal hyperplasia (all).
		Heart:	Mild multifocal chronic inflammation (11233, 11237, 11227). No significant lesions (others).
		Liver:	Chronic mononuclear pericholangitis, moderate (11233, 11227); with mild multifocal hepatic mononuclear infiltrates (11227). No significant lesions (others).
		Kidney:	Multifocal mononuclear chronic interstitial nephritis, moderate (11237), marked (11233), mild (11227). No significant lesions (others).

Discussion and Conclusion:

Pathologic changes are graded on a four-point scale: mild, moderate, marked, severe. All treated skin sections showed mild to marked degrees of one or more of the following changes: hyperkeratosis, acanthosis, adnexal hyperplasia, compared to untreated skin sections. There was no definitive dose related increase in severity of these skin lesions.

The incidence of hepatic fatty change was highest in both sexes of the intermediate dose group, and in males of the high dose group. The effect of the test material on this change was not clear.

Incidental renal lesions and cardiac lesions are unrelated to the treatment with test compound. Renal lesions of this kind, and secondary cardiac lesions, occur in rabbits routinely due to Encephalitozoon cuniculi, a common protozoon parasite of rabbits. Electron microscopy, however, would be necessary to show the existence of these parasites.

Liver lesions are due to the chronic proliferative cholangitis of the coccidian parasite Eimeria steidae, an ubiquitous parasite of laboratory rabbits. Granulomata are likewise of an incidental nature.

Intestinal lesions in low-dose male 11212 are considered secondary to stress and mucoid enteritis. Low dose male 11223 died having caught itself in its cage bars.

The test material caused skin changes after repeated contact. These changes were confined to thickening of the epidermal prickly cell layer, the outer horny layer and accessory cells of the dermis. Systemic toxicity was not evident.

Kirby N. Smith
T-4 Kirby N. Smith, DVM
Staff Pathologist

Table 21

DOSE LEVEL: 1000 mg/kg

TWO WEEK MULTIPLE DOSE DERMAL TOXICITY STUDY IN RABBITS OF
CI SOLVENT YELLOW 33

Rabbit Number	Sex	Body Weights (kg)					Food Consumption* (g)			
		Day 0	Day 3	Day 7	Day 10	Day 14	Day 0-3	Day 3-7	Day 7-10	Day 10-14
11213	M	3.0	3.0	3.1	3.3	3.4	657.9	855.9	692.5	844.0
11222	M	2.7	2.7	2.8	2.8	3.0	278.2	698.2	466.6	648.6
11221	M	2.6	2.6	2.7	2.6	2.6	437.2	534.2	421.8	397.1
11219	M	2.5	2.6	2.7	2.7	2.7	627.9	827.1	591.5	554.5
11207	M	2.4	2.4	2.5	2.4	2.5	545.1	712.1	524.6	557.0
Mean		2.6	2.7	2.8	2.8	2.8	509.3	725.5	539.4	600.2
S.D.		0.2	0.2	0.2	0.3	0.4	154.9	127.3	106.7	163.5
11230	F	2.7	2.7	2.9	3.0	3.1	574.3	742.7	646.3	617.7
11237	F	2.6	2.6	2.7	2.7	2.8	570.3	602.1	482.2	502.2
11233	F	2.5	2.3	2.6	2.6	2.7	368.6	506.7	428.7	508.4
11240	F	2.5	2.5	2.7	2.7	2.7	470.8	601.2	526.4	546.9
11227	F	2.3	2.0	2.0	2.4	2.3	357.5	295.6	428.5	476.1
Mean		2.5	2.4	2.6	2.7	2.7	468.3	549.7	502.4	530.3
S.D.		0.1	0.3	0.3	0.2	0.3	104.7	165.1	90.3	55.0

* Food consumption was measured on day 3, 7, 10 and 14 of the study.

Table 22

DOSE LEVEL: 200 mg/kg

TWO WEEK MULTIPLE DOSE DERMAL TOXICITY STUDY IN RABBITS OF
CI SOLVENT YELLOW 33

Rabbit Number	Sex	Body Weights (kg)					Food Consumption* (g)			
		Day 0	Day 3	Day 7	Day 10	Day 14	Day 0-3	Day 3-7	Day 7-10	Day 10-14
11204	M	2.7	2.8	3.0	3.0	3.2	623.2	815.3	527.8	715.6
11210	M	2.7	2.6	2.8	2.9	2.9	604.7	804.5	590.6	626.9
11214	M	2.6	2.6	2.8	2.8	2.9	515.1	653.6	481.8	583.4
11205	M	2.4	2.4	2.4	2.6	2.6	477.1	570.5	533.6	620.3
11208	M	2.4	2.6	2.8	2.8	2.9	602.6	709.2	545.9	588.3
Mean		2.6	2.6	2.8	2.8	2.9	564.5	710.6	536.0	626.9
S.D.		0.2	0.1	0.2	0.1	0.2	64.4	103.3	40.0	53.1
11224	F	2.9	2.8	2.9	3.0	3.1	586.5	734.0	502.6	501.5
11228	F	2.6	2.7	2.8	2.9	2.9	704.9	822.3	653.0	618.6
11242	F	2.6	2.6	2.8	2.8	2.8	503.6	541.2	566.5	459.4
11234	F	2.5	2.5	2.5	2.5	2.7	494.8	578.2	497.6	662.5
11239	F	2.4	2.4	2.6	2.5	2.7	507.8	687.4	460.0	635.4
Mean		2.6	2.6	2.7	2.7	2.8	559.5	672.6	535.9	575.5
S.D.		0.2	0.2	0.2	0.2	0.2	89.2	114.6	75.8	89.4

* Food consumption was measured on day 3, 7, 10 and 14 of the study.

Table 23

DOSE LEVEL: 50 mg/kg

TWO WEEK MULTIPLE DOSE DERMAL TOXICITY STUDY IN RABBITS OF
CI SOLVENT YELLOW 33

Rabbit Number	Sex	Body Weights (kg)					Food Consumption* (g)			
		Day 0	Day 3	Day 7	Day 10	Day 14	Day 0-3	Day 3-7	Day 7-10	Day 10-14
11215	M	3.0	2.9	3.1	3.1	3.1	650.5	850.7	690.7	683.0
11223	M	2.7	(a)	-	-	-	230.4	-	-	-
11216	M	2.5	2.5	2.6	2.7	2.6	562.3	675.4	473.9	417.3
11218	M	2.5	2.5	2.7	2.8	2.9	536.3	684.1	570.1	625.7
11212	M	2.4	2.4	1.9	-	-	457.5	142.4	2.3	-
	Mean	2.6	2.6	2.6	2.9	2.9	487.4	588.2	434.3	575.3
	S.D.	0.2	0.2	0.5	0.2	0.3	159.3	307.9	301.3	139.8
11232	F	2.7	2.7	2.9	2.9	3.1	664.0	787.2	601.4	802.2
11236	F	2.7	2.6	2.8	2.6	2.8	593.6	733.1	192.3	653.2
11243	F	2.6	2.6	2.8	2.8	2.9	528.0	727.4	578.3	746.6
11238	F	2.4	2.5	2.3	2.5	2.5	537.4	558.2	364.8	428.6
11241	F	2.4	2.5	2.5	2.6	2.7	635.9	622.3	524.1	673.5
	Mean	2.6	2.6	2.7	2.7	2.8	591.8	685.7	452.2	660.8
	S.D.	0.2	0.1	0.3	0.2	0.2	59.6	93.0	172.1	142.7

(a) - means animal died.

* Food consumption was measured on day 3, 7, 10 and 14 of the study.

Table 24

Two Week Multiple Dose Dermal Toxicity Study in Rabbits of CI Solvent Yellow 33

<u>Dose Level</u>	<u>Clinical Signs</u>			<u>Day of Observation</u>	<u>Median Time Sign First Observed</u>
	<u>Signs of Toxicity</u>	<u>Animal #</u>	<u>Sex</u>		
<u>1000 mg/kg</u>	mild diarrhea	11222	M	0	Day 0
		11207	M	2	Day 2
		11221	M	13	Day 13
<u>200 mg/kg</u>	mild nasal discharge	11213	M	2,4,7-9	Day 6
		11230	F	3	Day 3
	mild diarrhea	11224	F	7,8,10,11	Day 9
		11242	F	13,14	Day 14
		11228	F	14	Day 14
	moderate diarrhea	11224	F	14	Day 14
<u>50 mg/kg</u>	mild nasal discharge	11205	M	2,3,4	Day 3
	mild diarrhea	11218	M	14	Day 14

Table 25

Two Week Multiple Dose Dermal Toxicity
Study in Rabbits of CI Solvent Yellow 33Necropsy FindingsDose Level: 1000 mg/kg

<u>Animal #</u>	<u>Sex</u>	<u>Date and Time of Sacrifice</u>	<u>OBSERVATIONS</u>
11213	M	7/30/84 8:50 AM	No visible lesions
11222	M	7/30/84 8:50 AM	No visible lesions
11221	M	7/30/84 8:50 AM	No visible lesions
11207	M	7/30/84 8:50 AM	Scattered white lesions approx. 1 mm x 1 mm on lobes of liver.
11219	M	7/30/84 8:50 AM	No visible lesions
11227	F	7/30/84 9:00 AM	No visible lesions
11240	F	7/30/84 9:00 AM	No visible lesions
11233	F	7/30/84 9:00 AM	Petechial hemorrhages on both kidneys.
11237	F	7/30/84 9:00 AM	No visible lesions
11230	F	7/30/84 9:00 AM	No visible lesions

Table 26

Two Week Multiple Dose Dermal Toxicity
Study in Rabbits of CI Solvent Yellow 33Necropsy FindingsDose Level: 200 mg/kg

<u>Animal #</u>	<u>Sex</u>	<u>Date and Time of Sacrifice</u>	<u>OBSERVATIONS</u>
11210	M	7/30/84 9:15 AM	No visible lesions
11205	M	7/30/84 9:15 AM	Circular pattern (2 mm x 2 mm) of white circular lesions formed on medial liver lobe.
11208	M	7/30/84 9:15 AM	No visible lesions
11214	M	7/30/84 9:15 AM	Colon gaseous
11204	M	7/30/84 9:15 AM	Large intestine gaseous containing watery diarrhea.
11234	F	7/30/84 9:15 AM	No visible lesions
11224	F	7/30/84 9:15 AM	No visible lesions
11228	F	7/30/84 9:15 AM	No visible lesions
11242	F	7/30/84 9:15 AM	No visible lesions
11239	F	7/30/84 9:15 AM	No visible lesions

Table 27

Two Week Multiple Dose Dermal Toxicity
Study in Rabbits of CI Solvent Yellow 33

Necropsy FindingsDose Level: 50 mg/kg

<u>Animal #</u>	<u>Sex</u>	<u>Date and Time Found Dead or Sacrificed</u>	<u>OBSERVATIONS</u>
11223	M	7/18/84 8:00 AM	Animal found halfway out of cage, trapped between bars on cage. Subcutis and caudal thoracic and intercostal musculature: hemorrhages indicated by purple blotches in tissue. Thoracolumbar vertebrae column fractured at T13-L1.
11212	M	7/26/84 7:30 AM	Treated skin yellow. Multifocal suffusion hemorrhages on duodenum. Colon contains mucus, cecum wall is dark.
11216	M	7/30/84 12:45 PM	Colon gaseous
11218	M	7/30/84 12:45 PM	No visible lesions
11215	M	7/30/84 12:45 PM	No visible lesions
11238	F	7/30/84 12:45 PM	Both kidneys have scattered pale beige areas.
11241	F	7/30/84 12:45 PM	No visible lesions
11232	F	7/30/84 12:45 PM	Scattered white circular lesions (1 mm x 1 mm) over all lobes of liver.
11236	F	7/30/84 12:45 PM	Petechial hemorrhages and beige areas on both kidneys.
11243	F	7/30/84 12:45 PM	No visible lesions

Delayed-Type Contact Sensitization Study

CI Solvent Yellow 33

The body weight data, and evaluations of skin reaction for test and control animals are noted in Tables 28 and 29.

Sample 11357-9 when applied topically to the skin of ten guinea pigs produced very slight erythema to the test site of one guinea pig, 24 hours after removal of the patches. No irritation was visible in any of these animals one hour after removal of the patches. All of the test sites were stained gold from the test sample one hour after removal of the sample, and nine test sites appeared gold 24 hours after removal of the patches. The average irritation score for the sensitizing treatment (1 and 24 hour readings combined) for the test sample group was 0.05. Following the challenge application there was no erythema or edema visible on any of the test sites 1 or 24 hours after removal of the patches. All of the test sites were stained gold from the sample when observed 1 and 24 hours following removal of the patches after the challenge application. The average irritation score for the challenge treatment, for the test sample group, was 0.0. This sample did not produce sensitization upon topical application to guinea pigs.

The positive control sample, 0.1% 1-chloro-2,4-dinitrobenzene in 70% ethanol, when applied topically to the skin of ten guinea pigs, produced very slight erythema at the test sites of two guinea pigs one hour after removal of the patches. When observed 24 hours after removal of the patches, two guinea pigs exhibited very slight erythema of the test site. All of the test sites, for all ten animals, appeared yellow in color, 1 and 24 hours after removal of the patches. The average irritation score for the sensitizing treatment, for the positive control group, was 0.20. Following the challenge application very slight erythema was visible on the test site of seven guinea pigs, one hour after removal of the patches. One guinea pig exhibited well-defined erythema and very slight edema of the test site 1 and 24 hours after patch removal. Additionally, 24 hours after removal of the patches, five guinea pigs exhibited very slight erythema of the test site, and four animals exhibited well-defined erythema at the test site. Very slight edema was visible on the test site of four of these guinea pigs 24 hours after patch removal. All of the challenge test sites, for all ten guinea pigs, appeared yellow in color at both observation periods. The average irritation score for the challenge treatment, for the positive control group, was 1.5. The positive control sample did produce sensitization upon topical application to guinea pigs.

All of the guinea pigs, in both groups, gained weight throughout the study. The average weight gain for the test sample group was 186.4 grams, and the average weight gain for the positive control group was 203.1 grams.

TABLE 28
 DELAYED-TYPE CONTACT SENSITIZATION STUDY
 CI Solvent Yellow 33

<u>Animal Number</u>	<u>Sex</u>	<u>Body Weight (grams)</u> <u>Day 1</u>	<u>Day 37</u>
<u>TEST</u>			
2301	M	450	627
2306	M	449	683
2317	M	436	609
2305	M	431	578
2316	M	428	660
2314	M	426	612
2313	M	417	643
2307	M	408	588
2318	M	403	555
2304	M	389	546
<u>POSITIVE CONTROL</u>			
2311	M	450	625
2309	M	449	683
2312	M	448	676
2298	M	434	622
2315	M	431	607
2297	M	418	635
2308	M	416	664
2294	M	409	604
2296	M	404	609
2303	M	382	547

TABLE 29
 DELAYED-TYPE CONTACT SENSITIZATION STUDY
 CI Solvent Yellow 33

Observations: Erythema/Edema

<u>Animal Number</u>	<u>Sensitizing Treatment</u>		<u>Challenge Treatment</u>	
	<u>Day 1</u>	<u>Day 2</u>	<u>Day 36</u>	<u>Day 37</u>
TEST				
2301	0 ^a /0	0 ^a /0	0 ^a /0	0 ^a /0
2306	0 ^a /0	1/0	0 ^a /0	0 ^a /0
2317	0 ^a /0	0 ^a /0	0 ^a /0	0 ^a /0
2305	0 ^a /0	0 ^a /0	0 ^a /0	0 ^a /0
2316	0 ^a /0	0 ^a /0	0 ^a /0	0 ^a /0
2314	0 ^a /0	0 ^a /0	0 ^a /0	0 ^a /0
2313	0 ^a /0	0 ^a /0	0 ^a /0	0 ^a /0
2307	0 ^a /0	0 ^a /0	0 ^a /0	0 ^a /0
2318	0 ^a /0	0 ^a /0	0 ^a /0	0 ^a /0
2304	0 ^a /0	0 ^a /0	0 ^a /0	0 ^a /0
Average Score	0.05		0.0	
<u>POSITIVE CONTROL</u>				
2311	0 ^b /0	0 ^b /0	0 ^b /0	1 ^b /0
2309	0 ^b /0	0 ^b /0	1 ^b /0	2 ^b /1
2312	0 ^b /0	0 ^b /0	1 ^b /0	2 ^b /1
2298	1 ^b /0	0 ^b /0	1 ^b /0	1 ^b /0
2315	1 ^b /0	1 ^b /0	1 ^b /0	1 ^b /0
2297	0 ^b /0	0 ^b /0	1 ^b /0	2 ^b /1
2308	0 ^b /0	0 ^b /0	1 ^b /0	1 ^b /0
2294	0 ^b /0	1 ^b /0	0 ^b /0	2 ^b /0
2296	0 ^b /0	0 ^b /0	1 ^b /0	1 ^b /0
2303	0 ^b /0	0 ^b /0	2 ^b /1	2 ^b /1
Average Score	0.2		1.5	

^a Test site stained gold from sample
^b Treated site yellow

APPENDIX A

TEST METHODS FOR DERMAL AND EYE IRRITATION STUDIES

TEST METHODS

Primary Skin Irritants

Six albino rabbits are used to test each sample. Prior to application of the test sample, the hair is clipped from the sides in an area large enough to accommodate four 1 inch x 1 inch gauze pads (two on each side) without overlapping. The right anterior and left posterior of each rabbit is abraded by making four epidermal incisions, two parallel to the long axis of the rabbit and two at right angles to the first (bleeding should not be produced). Each animal is weighed on the day of dosing. A measurement of 0.5 grams of the test sample is introduced to each of four gauze pads, moistened with 0.9% sodium chloride and secured with surgical tape to the two abraded and two unabraded test sites. The trunk of the rabbit is wrapped in plastic wrap and then stockinette to prevent removal of the patches by the animal.

Twenty-four hours later, the patches are removed, excess test material is removed by wiping, and the resulting reactions in both abraded and both unabraded test sites are evaluated on the basis of the values in the table. Observations for erythema and edema are again made at 72 hours. Observations for irritation continues daily until all irritation subsides or is obviously irreversible. Responses are recorded at 7, 14, and 21 days if irritation persists for these periods. All clinical signs of toxicity are recorded during and after treatment.

Using the dual erythema and eschar/edema scales of Draize*, add the average value of erythema and eschar responses at 24 and 72 hours for intact skin to the average values on abraded skin at 24 and 72 hours (total of 4 values before addition). Similarly, add the values for edema formation at 24 and 72 hours for intact and abraded skin (4 values). The "value" recorded for each reading is the average value of the six or more animals subject to the test. The sum of the eight values is divided by 4 to give the Primary Irritation Score. Compounds producing primary irritation scores of 0 are non-irritating. Irritation scores greater than 0 and less than or equal to 0.5 are practically non-irritating. Primary Irritation scores of 0.6 to 2 are only mildly irritating; whereas those with indexes from 2.1 to 5.4 are moderate irritants, and those with scores above 5.5 are considered severe irritants.

ERYTHEMA AND ESCHAR FORMATION*

No erythema.....	0
Very slight erythema (barely perceptible).....	1
Well-defined erythema.....	2
Moderate to severe erythema.....	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth).....	4
TOTAL POSSIBLE ERYTHEMA SCORE.....	4

* Draize, J.H. 1965. Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics. Association of Food and Drug Officials of the U.S., Topeka, Kansas, pp. 46-59.

APPENDIX A (Continued)

TEST METHODS FOR DERMAL AND EYE IRRITATION STUDIES

EDEMA FORMATION*

No edema.....	0
Very slight edema (barely perceptible).....	1
Slight edema (edges of area well defined by definite raising).....	2
Moderate edema (raised approximately 1 mm).....	3
Severe edema (raised more than 1 mm and extending beyond area of exposure).....	4
TOTAL POSSIBLE EDEMA SCORE.....	4

Eye Irritants

Three albino rabbits are used to test each sample. Not more than 24 hours prior to treatment, all eyes are examined with an ophthalmoscope. Animals showing preexisting corneal injury or conjunctival irritation are eliminated. On the day of dosing, the animals are weighed, and a measurement of 100 mg (or an amount equal to 0.1 ml) of the test sample is applied on the everted lower lid of the right eye of each rabbit. The upper and lower lids are gently held together for 1 second before releasing to prevent loss of material. The left eye of each rabbit serves as the untreated control. The eyes are not washed following instillation of the test material. The treated eyes are observed for corneal opacity, hyperemia, chemosis and discharge at 24, 48, and 72 hours post-initiation of application according to the methods of Draize* (see table below). If injury persists, the eyes are observed and scored again on day 7, 14, and 21. All eye abnormalities not covered by the grading scale are recorded. All clinical signs of toxicity are recorded during and after treatment.

An animal is considered as exhibiting a positive reaction if the test sample produces at any of the readings ulceration of the cornea, opacity of the cornea, inflammation of the iris, or obvious swelling of the conjunctivae with partial eversion of the eyelids or a diffuse crimson color.

If the test material causes corrosion, severe irritation, or no irritation, then no further testing is performed. If equivocal responses occur, testing in 3 additional animals is performed.

*Draize, pp. 46-59.

APPENDIX A (Continued)

TEST METHODS FOR DERMAL AND EYE IRRITATION STUDIES*

CORNEA

A.	Opacity - degree of density (area most dense taken for reading)	
	No opacity.....	0
	Scattered or diffuse area, details of iris clearly visible.....	(1)**
	Easily discernible translucent areas, details of iris slightly obscured.....	2
	Opalescent areas, no details of iris visible, size or pupil barely discernible.....	3
	Opaque, iris invisible.....	4
B.	Area of cornea involved	
	One quarter (or less) but not zero.....	1
	Greater than 1 quarter, but less than half.....	2
	Greater than half, but less than three quarters.....	3
	Greater than three quarters, up to whole area.....	4
Score equals A x B x 5		Total Maximum = 80

IRIS

A.	Values	
	Normal.....	0
	Folds above normal, congestion, swelling, circumcorneal (any or all of these or combination of any thereof) iris still reacting to light (sluggish reactions are positive).....	(1)**
	No reaction to light, hemorrhage, gross destruction (any or all of these).....	2
Score equals A x 5		Total Maximum = 10

**Bracketed figures indicate lowest grades considered positive under Section 191.12 of the Federal Hazardous Substances Labelling Act Regulations.

*Draize, pp. 46-59.

APPENDIX A (Continued)

TEST METHODS FOR DERMAL AND EYE IRRITATION STUDIES*

CONJUNCTIVAE

- A. Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)
- Vessels normal.....0
- Vessels definitely injected above normal.....1
- More diffuse, deeper crimson red, individual vessels not easily discernible.....(2)**
- Diffuse beefy red.....3
- B. Chemosis
- No swelling.....0
- Any swelling above normal (includes nictitating membrane).....1
- Obvious swelling with partial eversion of the lids.....(2)**
- Swelling with lids about half closed.....3
- Swelling with lids half closed to completely closed.....4
- C. Discharge
- No discharge.....0
- Any amount different from normal (does not include small amounts observed in inner canthus of normal animals).....1
- Discharge with moistening of the lids and hairs adjacent to lids.....2
- Discharge with moistening of the lids and hairs, and considerable area around the eye.....3

Score equals (A + B + C) x 2

Total Maximum = 20

The maximum total score is the sum of all scores obtained for the cornea, iris, and conjunctivae. Total maximum score possible = 110.

**Bracketed figures indicate lowest grades considered positive under Section 191.12 of the Federal Hazardous Substances Labelling Act Regulations.

*Draize, pp. 46-59.

CLASSIFICATION OF TEST MATERIALS *
BASED ON EYE IRRITATION PROPERTIES

Rating	Range	Definition
Non-Irritating	0.0 - 0.5	To maintain this rating, all scores at the 24-hour reading must be zero; otherwise, increase rating one level.
Practically Non-Irritating	Greater than 0.5 - 2.5	To maintain this rating, all scores at the 24-hour reading must be zero; otherwise, increase rating one level.
Minimally Irritating	Greater than 2.5 - 15.0	To maintain this rating, all scores at the 48-hour reading must be zero; otherwise, increase rating one level.
Mildly Irritating	Greater than 15.0 - 25.0	To maintain this rating, all scores at the 96-hour reading must be zero; otherwise, increase rating one level.
Moderately Irritating	Greater than 25.0 - 50.0	To maintain this rating, scores at 7 days must be less than or equal to 10 for 60% or more of the animals. Also, the 7-day mean total score must be less than or equal to 20. If the 7-day mean total score is less than or equal to 20 but less than 60% of animals show scores less than 10, then no animal among those showing scores greater than 10 can exceed a score of 30 if rating is to be maintained, otherwise, increase rating one level.
Severely Irritating	Greater than 50.0 - 80.0	To maintain this rating, scores at 7 days must be less than or equal to 30 for 60% or more of the animals. Also, the 7-day mean total score must be less than or equal to 40. If the 7-day mean total score is less than or equal to 40 but less than 60% of the animals show scores less than or equal to 30, then no animal among those showing scores greater than 30 can exceed a score of 60 if rating is to be maintained, otherwise, increase rating one level.

Rating	Range	Definition
Extremely Irritating	Greater than 80.0 - 100.0	To maintain this rating, all scores at 7 days must be less than or equal to 60 for 60% or more of the animals. Also, the 7-day mean total score must be less than or equal to 80. If the 7-day mean total score is less than or equal to 80 but less than 60% of the animals show scores less than or equal to 60, then no animal among those showing scores greater than 60 can exceed a score of 100 if rating is to be maintained, otherwise, increase rating one level.
Maximally Irritating	Greater than 100.0 - 110	To maintain this rating, scores at 7 days must be greater than 60 for 60% of the animals. Also, the 7-day mean total score must be greater than 80. If the 7-day mean total score is less than or equal to 80 and less than 60% of the animals show scores greater than 60, then decrease rating one level. Otherwise, rating is to be maintained.

* Kay, J. H. and Calandra, J. C. (1962) J. Soc. Cos. Chem. 13, pp. 281-289.

APPENDIX B

TEST METHOD FOR ORAL TOXICITY STUDIES

TEST METHODS

Acute Oral LD50 Determination/Single Dose Oral Toxicity Test

An initial oral toxicity study on 5 male and 5 female F344 albino rats is performed at a dose of 5000 mg/kg body weight. All animals are fasted from food overnight prior to treatment. On the day of dosing, the animals were weighed and the dose per animal was based on the concentration of the test sample in the vehicle. The sample solution or suspension is administered in a single oral administration by gavage using a large ball tip needle attached to a plastic or glass syringe. Food is returned to the rats after dosing. All rats are observed frequently (at least 3 times) on the day of dosing, and twice daily thereafter for 14 days recording any deaths or signs of toxicity. The weight of dead animals is recorded as soon after death as possible. A complete gross necropsy is performed on all animals. The weight of each animal is determined at 3-4 day intervals throughout the test period.

All toxicologic and pharmacologic signs including nature, onset, severity, and duration of all abnormal or unusual cardiovascular, respiratory, excretory, behavioral, or other activity, as well as signs indicating an adverse effect on the the central nervous system (paralysis, lack of coordination, staggering); pupillary reaction; and time of death as near as possible are recorded. Animals were observed three times during the day of dosing, and twice daily thereafter for death and signs of toxicity. However, animals were observed only once on one day during the study due to a snow storm.

The acute oral LD50 determination is performed when mortality occurs upon administration of the test sample at 5000 mg/kg. For the LD₅₀ determination, 15 male and 15 female rats are used; 5 of each sex per dose group; 3 dose groups. All rats are randomized into dose groups to minimize bias and assure weight variation does not exceed $\pm 20\%$ of the mean weight. The procedure for this study is the same as for the initial single dose oral toxicity test.

APPENDIX C

TEST METHOD FOR ACUTE DERMAL TOXICITY STUDIES

TEST METHODS

Ten albino rabbits, 5 males and 5 females, are used to test each sample. Not more than 24 hours prior to treatment, all animals are clipped and examined. The clipped skin is further prepared by making epidermal abrasions over the area of exposure. On the day of dosing, the rabbits are weighed and the dose per animal is calculated. The powdered test sample is applied at a dose of 2000 mg/kg uniformly to a 6 inch x 6 inch pad, moistened with 0.9% sodium chloride, and placed over the dorsal surface area. The pad is secured to the exposure site with surgical tape. The trunk of the animal is wrapped in plastic wrap and then stockinette to prevent removal of the patches by the animal.

After the 24 hour exposure period, the patches are removed, and excess test material is gently wiped off the skin. Residual sample is removed by washing with cool water and blotted dry. Animals are observed at least 3 times on the day of dosing and at least twice daily thereafter for 14 days recording any deaths or signs of toxicity. All toxicologic and pharmacologic signs are recorded including time of onset, severity, duration, and time of death as near as possible. The weight of each animal is determined at 3-4 day intervals throughout the test period and at death as soon as possible. A complete gross necropsy is performed on all animals that die during the study or sacrificed at the termination of the study. Histopathologic examination of treated skin sites is performed on animals dying during the test period and on 2 animals per sex necropsied at the end of the test period.

APPENDIX D

TEST METHOD FOR REPEATED DOSE DERMAL TOXICITY STUDY

TEST METHODS

Thirty albino rabbits were used to test each sample. Prior to application of the test sample, the hair is clipped from the right side of each rabbit over at least 10% of the body surface area. All animals are randomized into 3 dose groups (5 males and 5 females/dose group) to minimize bias and assure weight variation does not exceed $\pm 20\%$ of the mean weight. On the first day of dosing, the animals are weighed and the dose per animal is calculated. The powdered test sample is moistened with 0.9% sodium chloride, applied uniformly over the area of exposure, and secured in place with surgical tape. The trunk of the animal is wrapped with plastic wrap and then stockinette to prevent removal of the patches by the animal. The test sample is kept in contact with the skin for 6 hours on each day of dosing. Animals are treated for 2 consecutive weeks, 5 days each week. Doses are recalculated on day 7 according to body weights. Body weights and food consumption are measured at 3-4 day intervals throughout the study.

All toxicologic and pharmacologic signs are recorded daily including their time of onset, intensity, and duration. Dermal irritation scores are recorded daily according to the method of Draize (1965)*, immediately prior to application of the test sample each day. A complete gross necropsy is performed on all animals that die during the course of the study. A complete gross necropsy is performed on the remaining animals at the termination of the test. Histopathologic examination on multiple skin sections of treated and untreated skin, all gross lesions, heart, liver, and kidney is performed on all animals which die during the test period and for all remaining animals at the termination of the test.

*Draize, J.H. 1965. Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics. Association of Food and Drug Officials of the U.S., Topeka, Kansas, pp. 46-59.

APPENDIX E

TEST METHOD FOR DELAYED-TYPE CONTACT SENSITIZATION STUDY

TEST METHOD

Twenty Duncan Hartley albino guinea pigs were used to test the sample. Prior to application of the test sample, the hair is clipped from a strip running from flank to trunk along the right side of each animal. This procedure is repeated throughout the test as necessary. All animals are randomized into two groups (10 for the sample, and 10 for the positive control) to minimize bias and assure weight variation does not exceed $\pm 20\%$ of the mean weight. On the first day of dosing, 0.5 grams of the powdered test sample was placed on a one inch gauze patch, moistened with water, and secured to the clipped area with surgical tape. The trunk of the animal is covered with plastic wrap, and then stockinette to prevent removal of the patch. The gauze patch is left in place for 6 hours and then removed. This procedure is followed 3 times weekly on alternate days for 3 weeks plus one additional day. A total of 10 applications shall be made. If during these sensitizing treatments, moderate to severe irritation occurs, the patch is placed on an unused site on the right side of the animal. The positive control sample (0.1% solution of 1-chloro-2,4-dinitrobenzene in 70% ethanol) is applied in the same manner as for the test sample.

After the last sensitizing treatment, the animals are given a two week rest period with no dosing. At the end of the two week rest period, a final challenge dose of 0.5 grams of the test or control sample is applied to a previously clipped site (unused) on the left side of each animal. The animals are treated in the same manner as for the 10 original treatments. Erythema, edema, and other lesions are scored at 1 and 24 hours after the first application and challenge application according to the methods of Draize (1965)*.

The average score from the first sensitizing treatment is calculated and compared to the average score from the challenge treatment. If the value for the challenge reading is substantially higher than for the average of the original readings, the sample can be considered to have produced sensitization.

*Draize, J.H. 1965. Appraisal of the Safety of Chemicals in Food, Drugs, and Cosmetics. Association of Food and Drug Officials of the U.S., Topeka, Kansas pp. 46-51.

APPENDIX F

Protocol Amendments and Deviations^(a)

TEST: Single Dose Oral Toxicity Test

PROTOCOL AMENDMENT:

Section 5.1.5 of the protocol should be amended to state that the age at start of study for the female rats is 61 to 150 days.

Section 5.1.6 of the protocol should be amended to state that the weight of the female rats at the start of the study is 150 to 225 grams.

TEST: Acute Oral LD₅₀ Determination

PROTOCOL AMENDMENT:

Section 5.1.5 of the protocol should be amended to state that the age of the female rats at the start of the study is 61 to 150 days.

Section 5.1.6 of the protocol should be amended to state that the weight of the female rats at the start of the study is 150 to 225 grams.

TEST: Primary Dermal Irritation Test

PROTOCOL AMENDMENT:

Section 6.5 of the protocol should be amended to state that all animals are weighed on the day of dosing prior to clipping.

TEST: Two Week Multiple Dose Dermal Toxicity Study

PROTOCOL AMENDMENT:

An addition to the protocol should be made to state that the doses are recalculated during the study according to day 7 body weight measurements.

TEST: Single Dose Eye Irritation Test

PROTOCOL AMENDMENT:

Section 6.3 of the protocol should be amended to state that not more than 24 hours prior to treatment, all eyes are examined using an ophthalmoscope.

(a) Amendments and Deviations apply to only those samples tested during Phase II.

APPENDIX F (continued)

Protocol Amendments and Deviations

TEST: Acute Dermal LD₅₀ Determination/Acute Dermal Toxicity Study in Rabbits

PROTOCOL AMENDMENT:

Section 6.5 of the protocol should be amended to state that not more than 24 hours prior to testing, animals shall have the hair carefully removed from their backs and sides by close clipping.

TEST: Single Dose Eye Irritation Test

PROTOCOL AMENDMENT:

Section 6.4 of the protocol should be amended to read as follows: "Weigh each animal on the day of treatment and record".

TEST: Single Dose Eye Irritation Test (Sample 11357-13)

PROTOCOL AMENDMENT:

Section 3.0 of the protocol should be amended for this sample to remove "If the test substance is corrosive or severely irritating in the dermal irritation test, the eye irritation test will not be performed".

REASON FOR CHANGE:

The Sponsor requested that these studies be performed at the same time.

TEST: Acute Oral LD₅₀ Determination/Single Dose Oral Toxicity Test (Samples 11357-12, 13 and 14)

PROTOCOL AMENDMENT:

Sections 6.6 and IV. 4. of the protocol should be amended to state that for nonaqueous liquids and suspensions, the volume administered orally to the rats should not exceed 2 ml/100 grams body weight.

Each test sample was suspended in corn oil at a concentration of 0.25 g/ml.

TEST: Acute Dermal, Eye, and Oral Toxicological Evaluations

PROTOCOL AMENDMENT:

Section II, A.5 of the protocol should be amended to state that Dr. Gunda Reddy is the Contracting Officer's Technical Representative (COTR).

APPENDIX F (continued)

Protocol Amendments and Deviations

TEST: Acute Oral LD₅₀ Determination (11357-13 and 14)

PROTOCOL AMENDMENT:

The doses to be used for the LD₅₀ determination as based on the acute oral range finding determination are as follows:

3300 mg/kg
1980 mg/kg
1188 mg/kg

Each test sample is suspended in corn oil at a concentration of 0.2 g/ml.

TEST: Acute Oral LD₅₀ Determination (Sample 11357-13 and 14)

PROTOCOL AMENDMENT:

Section 6.4 of the protocol should be amended to state that the animals are fasted from food overnight (20-24 hours) before treatment. Section 6.9 of the protocol should be amended to state that all animals shall be tested in the early afternoon over no more than a 3 hour period.

These changes have been implemented because of the delay in sample preparation and dosing.

TEST: Two Week Multiple Dose Dermal Toxicity Study (Sample 11357-9)

PROTOCOL AMENDMENT:

The doses to be used in this study are as follows:

1000 mg/kg
200 mg/kg
50 mg/kg

TEST: Delayed-Type Contact Sensitization Test (Sample 11357-9)

PROTOCOL AMENDMENT:

The Delayed-Type Contact Sensitization Test protocol module should be changed to read as follows:

- a. Section 5.2.2 "Positive: 0.1% (w/v) solution of 1-chloro 2,4-dinitrobenzene in 70% ethanol".

APPENDIX F (continued)

Protocol Amendments and Deviations

- b. Section 6.5 "The hair is removed by clipping from a strip running from flank to trunk along the right side of each animal. This procedure will be repeated throughout the test as necessary".
- c. Section 6.7 "The gauze patch is left in place for 6 hours before removal. This procedure is followed 3 times weekly on alternate days for 3 weeks plus one additional day. A total of 10 applications shall be made. If during the sensitizing treatments, moderate to severe irritation occurs, place the patch on an unused site on the right side of the animal. The positive control sample will be applied in the same manner as for the test sample".
- d. Section 6.9 "At the end of the 2 week rest period, a final challenge dose of 0.5 ml or grams of the test or control sample is applied to an untreated site on the left side of each animal. The animals are treated in the same manner as for the 10 original treatments".
- e. Section 6.10 "Erythema, edema and other lesions are scored at 1 and 24 hours after the first application and the challenge application according to Draize (1965)".
- f. Section 7.1 "Tabular data for each animal on scores for erythema and edema at 1 and 24 hours after the first sensitizing treatment and the challenge treatment".
- g. Section 7.2 "Calculate the average score from the first sensitizing treatment and the average score from the challenge treatment".
- h. Add Section 7.5 "If the value for the challenge reading is substantially higher than for the average of the original readings, the sample can be considered to have produced sensitization".

TESTS: Primary Dermal Irritation

PROTOCOL AMENDMENT:

Section 7.3 of the protocol should be amended to state that compounds producing primary irritation indexes of 0 is non-irritating; 0.1 to 0.5 is practically non-irritating; 0.6 to 2 are only mildly irritating; 2.1 to 5.4 are moderate irritants, and those with scores above 5.5 are considered severe irritants.

APPENDIX F (continued)

Protocol Amendments and Deviations

TESTS: Primary Dermal Irritation

PROTOCOL AMENDMENT:

Section 7.3 of the Primary Dermal Irritation protocol module should be amended to state that Primary Irritation Scores greater than 0 and less than or equal to 0.5 are practically non-irritating.

Reason For Change:

Protocol amendment #33 did not mention the category for scores between 0 and 0.1.

TESTS: Acute Dermal, Eye, and Oral Toxicological Evaluations

PROTOCOL AMENDMENT:

Section 5.1.12.2 of each protocol module should be changed to read as follows:

"Food: Agway Prolab R-M-H 3000

There are no contaminants that are reasonably expected to be present in the food at levels that are known to be capable of interfering with the purpose or conduct of the study".

Section 5.1.12.3 of each protocol module should be changed to read as follows:

"Water: Untreated from municipal water supply, ad libitum.

There are no contaminants that are reasonably expected to be present in the water at levels that are known to be capable of interfering with the purpose or conduct of the study".

TEST: All tests performed after 8/28/84

PROTOCOL AMENDMENT:

Amendment #26 should be amended to state that the changes indicated should apply to assays performed after 8/28/84, not samples received after 8/28/84.

APPENDIX F (continued)

Protocol Amendments and Deviations

TEST: Delayed-Type Contact Sensitization Test (Sample 11357-9)

PROTOCOL AMENDMENT:

Section 5.1.11 should be changed to read "Acclimatization Period" as opposed to Quarantine Period. Guinea Pigs for this study will be acclimatized in the same room with guinea pigs on similar studies.

TEST: All tests

PROTOCOL AMENDMENT:

BSC SOP entitled "Randomization Procedures for Rabbits or Large Rodents Using Body Weights" should be changed to "Randomization Procedures Using Body Weights" in the following places:

1. Dermal Irritation Test module section 6.1.
2. Acute Dermal LD₅₀ Determination/Acute Dermal Toxicity Study in Rabbits module section 6.2.
3. Acute Oral LD₅₀ Determination/Single Dose Oral Toxicity Test module section 6.3.
4. Delayed-Type Contact Sensitization Test module section 6.2.
5. Two Week Multiple Dose Dermal Toxicity Study in Rabbits module section 6.1.
6. Single Dose Eye Irritation Test module section 6.1.

TEST: Acute Dermal Toxicity Study in Rabbits (Samples 11357-8, 9 and 12)

PROTOCOL AMENDMENT:

The Acute Dermal Toxicity module should be amended to state that untreated skin will be evaluated histopathologically to be used as a comparison with the treated skin.

TEST: Primary Dermal Irritation Test and Acute Dermal LD₅₀ Determination/Acute Dermal Toxicity Study in Rabbits.

PROTOCOL DEVIATION:

Section 5.1.5 of the protocol has been deviated from in that it should read as follows: "Age at start of study: 8 to 15 weeks".

APPENDIX F (continued)

Protocol Amendments and Deviations

TEST: Two Week Multiple Dose Dermal Toxicity Study (Sample 11357-9)

PROTOCOL DEVIATION:

Section 6.11 of the Two Week Multiple Dose Dermal Toxicity Study has been deviated from in that only one section of untreated skin was evaluated and examined histologically.

TEST: Acute Oral LD₅₀ Determination (Sample 11357-13 and 14)

PROTOCOL DEVIATION:

Section 6.4 of the Acute Oral LD₅₀ Determination module has been deviated from in that animals were fasted longer than stated in the protocol.

TEST: Delayed - Type Contact Sensitization Test (Sample 11357-9)

PROTOCOL DEVIATION:

Section 5.1.11 of the protocol was deviated from in that the guinea pigs were acclimatized to the facilities for more than one week.

TEST: Acute Oral LD₅₀ Determination (Sample 11357-13 and 14)

PROTOCOL DEVIATION:

Section 6.8 of the protocol has been deviated from in that mortality rates between 10% and 90% bracketing the expected LD₅₀ was not produced. The Sponsor requested that another LD₅₀ not be performed due to the nature of the sample and its suspendibility.

TEST: Single Dose Eye Irritation Test (Sample 11357-13)

PROTOCOL DEVIATION:

For the Single Dose Eye Irritation Test, extra animals from the Two Week Multiple Dose Dermal Toxicity Study were used. No actual randomization occurred.

TEST: Single Dose Eye Irritation Test (Sample 11357-8, 9 and 12)

PROTOCOL DEVIATION:

There was no documentation that the amount of the powdered samples administered to the eyes of rabbits had a volume of 0.1 ml weighing not more than 100 mg.

APPENDIX F (continued)

Protocol Amendments and Deviations

TEST: Primary Dermal Irritation Test (Sample 11357-12)

PROTOCOL DEVIATION:

Animals were randomized using a random numbers table, not according to body weight as originally stated in the protocol.

TEST: Acute Oral LD₅₀ Determination/Single Dose Oral Toxicity Test

PROTOCOL DEVIATION:

Section 6.4 of the Acute Oral LD₅₀ Determination module has been deviated from in that animals were fasted longer than stated in the protocol.

TEST: Acute Oral Range Finding Determination

PROTOCOL AMENDMENT:

Section II, A.5 of the protocol should be amended to state that Dr. Gunda Reddy is the Contracting Officer's Technical Representative (COTR).

TEST: Acute Oral Range Finding Determination (11357-13 and 14)

PROTOCOL AMENDMENT:

- a) Section 5.1.4 of the protocol should be amended to state that the rats are supplied by Taconic Farms, Germantown, NY.
- b) The dose levels to be used in this test are as follows:

- 3500 mg/kg
 - 1050 mg/kg
 - 315 mg/kg
 - 94.5 mg/kg

The test sample is suspended in corn oil at a concentration of 0.15 g/ml.

TEST: Acute Oral Range Finding Determination (11357-13 and 14)

PROTOCOL AMENDMENT:

Section 6.4 of the protocol should be amended to state that the rats will receive no more than 3 ml/100 grams of body weight of the test sample suspension.

APPENDIX F (continued)

Protocol Amendments and Deviations

TEST: Acute Oral Range Finding Determination (11357-13 and 14)

PROTOCOL AMENDMENT:

The protocol should be amended to state that animals dying during the study are necropsied for gross visible lesions.

TEST: Acute Oral Range Finding Determination

PROTOCOL AMENDMENT:

The protocol should be amended to add that the results of the Acute Oral Range Finding Determinations will be reported in the final report with assays performed under the Acute Dermal, Eye, and Oral Toxicological Evaluations protocol.

TEST: Acute Oral Range Finding Determination (11357-13 and 14)

PROTOCOL DEVIATION:

Section 6.5 of the protocol has been deviated from in that animals were fasted longer than the 18 hours specified in the protocol.

Section 6.7 of the protocol has been deviated from in that variability in test volumes was not minimized by adjusting concentration.

APPENDIX G

QA Inspections

Primary Dermal Irritation Study

<u>Phase</u>	<u>Sample(s)</u>	<u>QA Inspection</u>	<u>Findings Reported to Technical Supervisor/Study Director</u>	<u>Findings Reported to Management</u>
Protocol Review	11357-1-19	10/13/82	10/13/82	11/23/82
Test Article Admin.	11357-8	08/09/83	08/09/83	09/27/83
Data Observations	11357-8	08/10/83	08/10/83	09/27/83
Test Article Admin.	11357-12	09/07/83	09/09/83	10/04/83

Acute Dermal Toxicity Study

<u>Phase</u>	<u>Sample(s)</u>	<u>QA Inspection</u>	<u>Findings Reported to Technical Supervisor/Study Director</u>	<u>Findings Reported to Management</u>
Protocol Review	11357-1-19	10/13/82	10/13/82	11/23/82
Data Observations	11357-13	12/23/83	12/23/83	06/03/84
Necropsy	11357-12	02/13/84	02/13/84	06/03/84

Primary Eye Irritation Test

<u>Phase</u>	<u>Sample(s)</u>	<u>QA Inspection</u>	<u>Findings Reported to Technical Supervisor/Study Director</u>	<u>Findings Reported to Management</u>
Protocol Review	11357-1-19	10/13/82	10/13/82	11/23/82
Test Article Admin.	11357-13	12/20/83	12/21/83	05/31/84
Data Observations	11357-13	12/23/83	01/03/84	06/03/84
Data Observations	11357-13	06/07/84	06/07/84	06/08/84

QA Inspections (continued)

Single Dose Oral Toxicity Test

<u>Phase</u>	<u>Sample(s)</u>	<u>QA Inspection</u>	<u>Findings Reported to Technical Supervisor/Study Director</u>	<u>Findings Reported to Management</u>
Protocol Review	11357-1-19	10/13/82	10/13/82	11/23/82
Dose Preparation	11357-13,-14	01/19/84	01/23/84	06/03/84
Test Article Admin.	11357-12,-13,-14	01/19/84	01/23/84	06/03/84

Two Week Multiple Dose Dermal Toxicity Study

<u>Phase</u>	<u>Sample(s)</u>	<u>QA Inspection</u>	<u>Findings Reported to Technical Supervisor/Study Director</u>	<u>Findings Reported to Management</u>
Protocol Review	11357-1-19	10/13/82	10/13/82	11/23/82
Food Consumption	11357-9	06/07/84	06/07/84	06/08/84
Test Article Admin.	11357-9	07/18/84	07/18/84	07/20/84
Data Observations	11357-9	07/18/84	07/18/84	07/20/84

Acute oral LD50 Determination

<u>Phase</u>	<u>Sample(s)</u>	<u>QA Inspection</u>	<u>Findings Reported to Technical Supervisor/Study Director</u>	<u>Findings Reported to Management</u>
Protocol Review	11357-1-19	10/13/82	10/13/82	11/23/82
Data Observations	11357-13	05/30/84	05/31/84	06/03/84

QA Inspections (continued)

Delayed-Type Contact Sensitization

<u>Phase</u>	<u>Sample(s)</u>	<u>Findings Reported to Technical Supervisor/Study Director</u>		<u>Findings Reported to Management</u>
		<u>QA Inspection</u>	<u>QA Inspection</u>	
Protocol Review	11357-19	10/13/82	10/13/82	11/23/82
Data Observations	11357-9	09/18/84	09/19/84	09/21/84
<u>Raw Data and Draft Report Review</u>				
	<u>Sample(s)</u>	<u>Findings Reported to Technical Supervisor/Study Director</u>		<u>Findings Reported to Management</u>
		<u>QA Inspection</u>	<u>QA Inspection</u>	
Primary Dermal Irritation Raw Data	11357-8,9	03/06/85	03/06/85	04/08/85
Primary Dermal Irritation Raw Data	11357-12,-13,-14	03/05/85	03/05/85	04/08/85
Acute Dermal Toxicity Study Raw Data	11357-8,-9,-12	03/05/85	03/05/85	04/08/85
Primary Eye Irritation Test Raw Data	11357-8,-9,-12,-13,-14	03/05/85	03/05/85	04/08/85
Primary Eye Irritation Test Raw Data	11357-13	03/06/85	03/06/85	04/08/85
Single Dose Oral Toxicity Raw Data	11357-8,-9,-12,-13,-14	03/05/85	03/05/85	04/08/85
Two Week Multiple Dose Dermal Toxicity Raw Data	11357-9	03/06/85; 03/11/85	03/06/85; 03/11/85	04/08/85
Acute Oral LD50 Determination Raw Data	11357-13,-14	03/06/85	03/06/85	04/08/85

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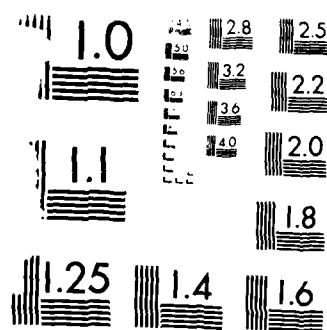
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QA Inspections (continued)

Raw Data and Draft Report Review

	<u>Sample(s)</u>	<u>QA Inspection</u>	<u>Findings Reported to Technical Supervisor/Study Director</u>	<u>Findings Reported to Management</u>
Acute Oral Range Finder Determination Raw Data	11357-13,-19	03/06/85	03/06/85	04/08/85
Delayed-Type Contact Sensitization Raw Data	11357-9	03/04/85	03/04/85	04/08/85
Chemical Preparation and Usage Raw Data	11357-8,-9,-12, -13,-14	03/07/85	03/07/85	04/08/85
Draft Report Review	11357-8,-9,-12, -13,-14	03/13/85; 03/20/85	03/20/85	04/08/85
Draft Report Review	11357-8,-9,-12	06/11/85; 06/12/85	06/12/85	06/18/85
Final Report Review	11357-8,-9,-12, -13,-14	02/19/86	02/20/86	02/26/86

Paul S. Selinger
Quality Assurance Specialist 2/26/86 Date

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